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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

The Microscopic Changes in the Nervous System in a Case of Chronic Dourine or Mal de Coit, *and Comparison of the Same with those Found in Sleeping Sickness.*

By F. W. Mott, M.D., F.R.S.

(Received February 21,—Read March 8, 1906.)

(From the Pathological Laboratory of the London County Asylums, Claybury.)

[Plates 1—4.]

Introduction.—I am indebted to Dr. Lingard, of the Imperial Bacteriological Laboratory of India, for the nervous tissues of an Arab stallion which acquired Dourine May 4 to 6, 1903. It exhibited 156 cutaneous plaques together with marked symptoms of paraplegia, and died August 15, 1905, 27½ months after infective coitus.

This disease, Dourine, is due to a specific form of trypanosome which has the power of penetrating the mucus membrane, affects equines, and is transmitted like syphilis by coitus. This is of especial interest, since Schaudinn has demonstrated the *Spirochæta pallida* of syphilis, particularly as it seems possible that trypanosomes may undergo a spirillar modification.

It is also of interest because, like some other trypanosome infections, it may, and frequently does, run a very chronic course and, as in the case under consideration, more than two years may elapse before a fatal termination. Again the lesion found in the lumbo-sacral region of the spinal cord presents some points of resemblance to a localised syphilitic meningo-myelitis.

A comparative examination of the nervous tissues in this disease with that of animals infected with *Trypanosoma Gambiense,* and with the tissues of human beings dying of chronic Sleeping Sickness, especially those in which there was no evidence of terminal or secondary microbial infection, is of interest in showing that prolonged trypanosome infection causes in all three conditions a marked proliferation and overgrowth of the subpial, septal, and perivascular neuroglia tissue. A chronic interstitial inflammation of the connective tissue structures with lymphocyte infiltration occurs, owing to the presence of an irritative agency in the lymphatic system, which, in the case of Dourine, starting in *one* seat of primary infection, extends to the inguinal glands, thence presumably by the pelvic lymphatics to the lumbo-sacral plexus and the posterior lumbo-sacral roots to the central nervous system ; consequently the lower part of the spinal cord and especially the posterior column is first and most affected. In the case of Sleeping Sickness there may be any number of seats of infection, but the cervical glands are nearly always markedly involved.

Material and Notes of Case.—The following portions of the central nervous system of the stallion, hardened in Formol-Muller solution, were examined by various methods to display the neural and neuroglial structures. (1) Brain, lateral side of left lobe. (2) Portion of cervical spinal cord, between third and fourth cervical nerves. (3) Portion opposite the 19th nerve (12th dorsal). (4) Portion taken midway between the 22nd and 23rd pair of nerves (15th and 16th dorsal). (5) Portion with 30th pair of nerve roots attached.

The notes accompanying these tissues were as follows :—

An account of the Arab stallion (Monarch) will be found in the Appendices, "Report on Dourine in Different Breeds of Equines, etc.," by Alfred Lingard, M.B., M.S., D.P.H., Imperial Bacteriologist to the Government of India. Page 21.—Infective coitus occurred on May 4 to 6, 1903. Eruption of 156 cutaneous plaques, between June 6, 1903, and August, 1905. Partial paraplegia appeared February 25, 1904. Death (836th day) August 15, 1905.

Post-mortem.—A considerable quantity of gelatinous exudation was found round the lumbar portion of the spinal cord, and a smaller amount around the cervical enlargement, and a certain quantity of cerebro-spinal fluid escaped from within the membranes on removal.

The cerebro-spinal fluid did not exhibit the *Trypanosoma Equiperdum* when searched for in numerous stained specimens.

Previous Observations on the Changes in the Nervous System in Dourine.

It is unfortunate that nerves of the hinder extremities were not sent, for Laveran et Mesnil* thus refer to the histological examination by Marck: "He showed a degeneration of the nerve fibres of the posterior columns; the other parts of the spinal cord (grey substance and other bundles of white substance) are in a healthy state. Some nerve fibres, especially on the sensory side, are degenerated at different points; the nerves of the fore-limbs are less altered. Having ascertained these facts Marck calls Dourine infective polyneuritis of the horse."

Methods of Examination.—Some portions of the tissues were embedded in paraffin and sections cut 10 μ thickness and stained by the following methods:—Polychrome and Eosin, Azure Blue, Van Gieson and Leishman's stain. Other portions were embedded in celloidin and sections of 20 μ thickness were cut and stained by the new Weigert method, modified Mallory and by Van Gieson's fluid. The sections by this method were thicker, but I was enabled to obtain sections of uniform thickness of the cord and membranes together with the roots, inflammatory material and attached vessels.

Description of Histological Changes in Dourine.

Throughout the grey matter of the spinal cord the ganglion cells show marked chromolytic changes and the vessels exhibit evidence of chronic inflammation with scattered capillary hæmorrhages.

The small vessels show lymphocyte infiltration around, but there is nothing resembling the marked perivascular lymphatic infiltration met with *throughout* the grey matter in all cases of well-marked Sleeping Sickness. The ganglion cells for the most part retain their normal outlines, but are stained a uniform bluish purple with a badly defined and imperfectly stained pattern of Nissl granules. (*Vide* Photomicrograph 1.) The most marked change is observed in the lumbar region.

Sections of the lumbo-sacral cord with attached roots, after embedding in celloidin and staining with Van Gieson's fluid and by the Weigert method, exhibited the following changes. The roots, anterior and posterior, are infiltrated with lymphocytes, also all the vessels are surrounded and their walls infiltrated with small round cells. The connective tissue septa carrying the vessels as well as the perineurion and endoneurion are thickened and infiltrated with lymphocytes, also the loose connective tissue outside the dura mater; the dura mater itself and the vessels and tissues in the subdural space show signs of chronic inflammation. The condition simulates an acute syphilitic meningitis in many ways, except that I can discover only occasional evidence of an obliterative arteritis. (*Vide* figs. 1 and 2, Plate 2 and Photomicrograph 12.)

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* 'Trypanosomes et Trypanosomiasis,' p. 283.

Some of the larger roots seen in the section (*vide* Photomicrograph 7) are very markedly affected by the inflammatory process. These are judged to be posterior roots, because a posterior spinal ganglion cell can be seen here and there in them; moreover, they occupy among the roots a posterior position. The capsules of the ganglion cells that are seen are crowded with lymphocytes presenting an appearance like that observed in Sleeping Sickness. Some of these roots in transection under a high power show the nerve fibres to have been destroyed and their place occupied by proliferated branching neurilemmal connective tissue cells lying in the centre of an oval or circular space bounded by highly vascular thickened, and swollen and amorphous endoneurion. In the centre of most of these cells is a highly refractive round or oval space. (*Vide* fig. 6, Plate 1.)

Throughout the spinal cord, but especially at the lumbo-sacral and cervical enlargements, there is a marked thickening of the subpial network of the glia tissue which extends into the white substance along the main septa and branches. (*Vide* Plate 2, fig. 2.)

At the periphery the proliferated glia tissue consists mainly of a dense reticulum of fibrils, but in the substance of the white matter great numbers of large branching neuroglia cells are seen sending their processes in all directions. On careful examination of the longitudinal and transverse sections (*vide* Photomicrographs 5 and 6), these proliferated neuroglia cells, which are often spoken of as mesoglia cells, can be seen to send their processes to end like a foot upon the wall of a small vessel. This overgrowth of glia tissue is seen throughout the white matter of the spinal cord whatever region is examined, but more especially in the lumbar and cervical enlargements, especially the former. It is more obvious in the posterior columns than elsewhere, especially along the median fissure and in the root zone. It does not *wholly* correspond to system tracts of fibres which have undergone degeneration, but appears (except in the root zone of the posterior column) to be a chronic formative proliferation of the glia tissue caused by an irritant entering the lymphatics and subarachnoid space. The posterior column is much more affected than the rest of the white matter.

In the lumbo-sacral region there are three definite zones of degeneration in the posterior column, no doubt corresponding to the destroyed roots. (*Vide* Photomicrograph 8.) The lymphocyte infiltration is observed around the small vessels and numerous lymphocytes are scattered about in the septa of the white matter. In the roots a well-marked perivascular infiltration with lymphocytes can be seen and some hæmorrhages in the lumbar region. The chronic interstitial inflammation of the anterior roots, in which all the nerve fibres appear to be normal, together with the fact that there is subpial proliferation of the glia tissue of the lumbo-sacral and cervical enlargements in their entire circumference, with extension of the same along the septa, shows that this proliferation of the connective tissue supporting structure, whether of the undamaged roots or of the spinal cord, is not due to atrophy of the nervous elements; although in the posterior columns where there are three definite bands of sclerosis the neuroglial proliferation is without doubt secondary to neural destruction. (*Vide* Photomicrograph 3.) Occasional foci of micro-organisms are seen, but do not play any part in the *chronic* changes above described. They are not found in the blood or inflammatory exudations. In none of the sections could I find any trypanosomes stained by the various methods, which we know will show them, if they are present in any numbers. Since the above description was written I have received from Dr. Lingard the remainder of the central nervous system, including a number of the spinal ganglia. *I was thus enabled when reading the paper at the Royal Society on March* 8 *to communicate the following additional facts*, which have, I consider, an important bearing upon the degeneration of the posterior roots and the sclerosis in the posterior columns. The facts which will be now stated may also explain some of the characteristic symptoms of the disease and afford some further evidence of similarity to Sleeping Sickness in the morbid change occasioned by chronic trypanosome infection.

Sections of the posterior spinal ganglia and attached roots in the cervical, upper dorsal, mid dorsal, lower dorsal, and lumbo-sacral regions have been examined by the methods previously described and the appearances compared with those observed in Sleeping Sickness. In all these ganglia there was evidence of intense chronic inflammation with marked proliferation of the endothelial nuclei of the capsule and lymphatics, together with lymphocyte infiltration of the interstitial fibrous tissue, and this morbid change can be followed from the nerves to the ganglion and along the posterior roots. This change is most marked in the lower dorsal and lumbo-sacral ganglia, and where the chronic inflammation is most intense, there the posterior spinal ganglion cells are most affected. In all the sections some of the ganglion cells have undergone vacuolar degeneration, and even complete destruction, their place being occupied by inflammatory products (*vide* Photomicrograph 4), but the neuronic destruction is most marked in the lumbar region, which is the situation, as before remarked, of extensive posterior root destruction and system-degenerative sclerosis of the posterior columns. (*Vide* Photomicrograph 3.)

Both these conditions are the outcome of the destruction of the posterior spinal ganglion cells. But this destruction must also have led to destruction of the peripheral branch of the T-shaped process of the ganglion cells, and this would give rise to a sensory polyneuritis. Unfortunately, I have not had forwarded to me any of the nerves to examine. However, the comparatively normal appearance of the anterior roots, and the very complete destruction of many of the sensory roots, together with the well-marked sclerosis of the posterior columns, would suggest that this animal may have suffered with a sensory paralysis of the hind limbs analogous to tabes dorsalis, rather than a polyneuritis. An argument in favour of this hypothesis is that in severe alcoholic and other forms of polyneuritis the motor anterior horn cells usually show characteristic degenerative changes which are not seen in the spinal cord of this animal. Now it has been shown by numerous authorities, but especially in a very systematic manner by Head and Campbell, that herpes zoster is caused by an inflammation of the posterior spinal ganglia, the seat of the eruption depending upon the particular segmental ganglion or ganglia affected. It is therefore reasonable to associate the eruption of the characteristic cutaneous plaques with the inflammatory irritation of the ganglia as they become successively affected by the noxious agent.

Both Lingard and Laveran remark upon the curious nature of the eruption: the former believes it to be an angio-neurotic œdema which occurs in the form of circular plaques, as if a ring had been introduced under the skin. They remark that although trypanosomes can only be found in the blood with difficulty, yet they are always present in the fluid which can be drawn from a plaque. Lingard concludes therefore that embolism by trypanosomes is the cause, but if there is an angio-neurotic œdema occasioned by the irritation of the posterior spinal ganglia, then it is possible that in the blood or the inflammatory exudation the trypanosomes may find suitable conditions for multiplying by fission. The theory which I have advanced for the origin of the rash finds some support, moreover, in experiment, for Dr. Bayliss has shown that stimulation of the posterior roots produces vaso-dilation. Again, these plaques often leave patches of leucoplacia which may be due to neurotrophic causes associated with the destruction of numbers of the spinal ganglion cell neurotrophic centres.

In chronic trypanosome infections by *T. Gambiense*, even before the lethargy occurs, outbursts of irritative papules or other skin eruptions occur, and they might be accounted for by irritation of the neurotrophic centres in the spinal ganglia. The changes in the ganglia are never so intense as in

Dourine, but I have now examined quite a number of posterior spinal ganglia in Sleeping Sickness cases, and I have never failed to find some change, never sufficient, however, to produce cell destruction, such as is found in Dourine. The most intense change I have met with is shown in Photomicrographs 7 and 8, and this in no way differs, except in degree, from that seen in Dourine.

Laveran and Mesnil mention that dislocation and fractures may occur in Dourine, and we know that spontaneous dislocation and fractures are met with in tabes dorsalis, a disease in which the posterior roots and posterior columns of the spinal cord undergo degenerative atrophy.

The membranes at the base of the brain of this animal, which died of Dourine, seemed thicker than normal. On microscopic examination sections of the peduncles and interpeduncular structures exhibited a subpial and septal neuroglia proliferation very similar to that seen in the lumbar region of the spinal cord. I observed, however, but little or no lymphocyte infiltration.

Comparison of the Changes in the Tissues of Animals Dying after Inoculation with Trypanosoma Gambiense, *also with the Tissues of Human Sleeping Sickness, with those met with in the Case of Dourine above described.*

The examination of the tissues of the spinal cords of a number of cases of Sleeping Sickness, especially of several chronic cases in which there was no terminal or secondary infection from suppurating glands, shows a glia proliferation in the spinal cord and throughout the nervous system similar to that above described, and it may be remarked that the lymphocyte proliferation is not always in proportion to the glia proliferation. Moreover, in all chronic cases there is a marked glia proliferation around the central canal of the cord.

Zururu bin Mza and Masake* were very chronic cases in which there was a most extensive glia proliferation certainly not all due to secondary degeneration, but caused by a chronic interstitial inflammatory extension along the subpial septa and perivascular spaces (*vide* figs. 1 and 2, Plate 1) without marked destruction of the intervening nerve fibres.

"Cases of two monkeys inoculated with *Trypanosoma Gambiense*"†:—

(1) A monkey infected by fresh fly feeding, the animal dying eight months later. No perivascular lymphocyte infiltration was discovered in the central nervous system, but there was a very marked chromolytic change in the ganglion cells of the medulla, also a considerable degree of acute glia cell

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* *Vide* 'Reports of the Sleeping Sickness Commission,' No. VI, pp. 270 and 234.

† *Vide* 'Reports of the Sleeping Sickness Commission,' No. VI.

proliferation. There was also in the subjacent cortical white matter a perivascular glia proliferation. (*Vide* figs. 3 and 4, Plate 1, and Photomicrograph 12.)

(2) The monkey dying eighteen months after inoculation with *Trypanosoma Gambiense*, in which the lesion of Sleeping Sickness was found by Major Leishman and described by Captain Harvey.*

I have found in portions of the brain of this animal (kindly given to me by Major Leishman) a profound neuroglia proliferation, similar to that seen in Dourine and chronic Sleeping Sickness. The lymphocyte proliferation is not very marked, and the cellular proliferation in the perivascular lymphatics is in many parts due more to neuroglial proliferation than lymphocyte infiltration. Photomicrograph 11 and fig. 5, Plate 1, show this proliferation in the sheath of a vessel which has been cut longitudinally. This seems to indicate the pathology of the process, viz., the overgrowth of the perivascular glial tissue meshwork, which stops the proliferated lymphocytes, and leads to their accumulation in the perivascular sheath.

Conclusions.

The more chronic the case of trypanosome infection, the more extensive is the glia cell proliferation. The overgrowth of glia tissue is manifested by an increase in number and size of cells and fibrils. Many large cells are seen with branching processes, sending one process to form a foot on the vessel wall, others are seen entering into the formation of a dense reticulum in the perivascular lymphatic space. (*Vide* Plate 1, figs. 1 and 5, and Photomicrographs 10, 11, 12.) Normally, the perivascular spaces have only a few delicate septa passing across, which could not in any way impede the flow of the cerebro-spinal fluid and lymphocytes.

Dr. Eisath, working in my laboratory, has recently shown by a differential method of staining that a large number of the round cellular elements contained in the perivascular infiltration of chronic Sleeping Sickness are not lymphocytes, but the nuclei of neuroglia cells. I have not found this glial proliferation in acute cases of trypanosome disease of animals, only in the chronic case of Dourine, and in the two cases of experimental Sleeping Sickness in monkeys above referred to. The glial proliferation in these cases is in excess of, and appears to precede, the perivascular lymphocyte accumulation so constantly found in human Sleeping Sickness, which we now know may be a very chronic disease. Moreover, in most cases of uncomplicated human Sleeping Sickness, there is in the spinal cord a universal proliferation of the glia tissue extending along the subpial septa and vessels, without apparently destroying the nerve fibres. (*Vide* Photomicrograph 9.)

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* "Report on a Case of Experimental Sleeping Sickness in a Monkey, *Macacus rhesus*," 'Journal of the Royal Army Medical Corps,' No. 5, vol. 4.

It is possible, therefore, that the lymphocyte accumulation in the perivascular lymphatics does not occur until the neuroglia proliferation has become advanced, and this may account for the fact that leptomeningeal perivascular infiltration with lymphocytes (so-called chronic meningo-encephalitis) is not met with in the nervous tissues of animals inoculated with various trypanosomes, that die within a comparatively short time, either from exhaustion, or from the poisonous effects of the infection upon the cells of the nervous system or from secondary microbial invasion.

Greig* concludes from his experiments that the onset of the symptoms of Sleeping Sickness synchronise with the entrance of the trypanosomes into the lymph spaces of the nervous system, and this is accompanied by an increase of lymphocytes in the cerebro-spinal fluid. Whether it be as Greig's experiments suggest, that the continuous presence of the trypanosomes in the cerebro-spinal fluid acts as a direct cause of irritation of the lymphatic structures of the central nervous system; or whether it be the result of the absorption of toxic products from the multiplication of the trypanosomes in the lymphatic glands; or whether it be the extension of a chronic inflammatory process from the paravertebral glands along the lymphatics of the vessels and nerves proceeding to the cerebro-spinal axis, as seems probable in Dourine, one fact is certain, and that is, the symptoms of Sleeping Sickness are associated with, and the depth of the lethargy and chronicity of the disease is in great measure proportional to, the extent and degree of leptomeningeal, septal, and perivascular lymphatic cell proliferation. This cell proliferation is made up partly by an overgrowth both in number and size of the neuroglial cells to form a dense meshwork and partly of accumulated entangled and occasionally altered lymphocytes. In proportion to this chronic lymphatic inflammation the neural elements display relatively slight degenerative changes in cases uncomplicated by microbial infection; thus differing from Dourine, where there was very obvious degeneration of posterior spinal ganglion cells and roots with corresponding degeneration in the posterior column.

The mind of a patient suffering with Sleeping Sickness remains clear usually until near the end. He can easily be aroused from his lethargy, and comprehends what is said to him, replying in a slow, sleepy manner, and with manifest disinclination to continue a conversation, or to exercise any mental or bodily effort. These symptoms point to a functional depression of the anatomical basis of the seat of consciousness rather than to a widespread destruction as in general paralysis of the insane. This functional depression of the neural elements may be accounted for, by (*a*) increased intracranial pressure brought about by the retardation of the lymph circulation and the cell proliferation; (*b*) the interference with the blood circulation, and with the gaseous and metabolic exchanges between the blood and the lymph, and between the lymph and the neurone; (*c*) in chronic cases by a certain amount of neurone destruction as shown by Marchi's degeneration of fibres, and a certain amount of cell and fibre destruction.

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* 'Reports of the Sleeping Sickness Commission,' Vol. 6.

DESCRIPTION OF PLATES.

Plate 1.

In these figures only the neuroglial tissue is shown. Magnification × 500.

Fig. 1.—Proliferating branching glia cells in the subpial tissues of the cortex in a case of very chronic uncomplicated human Sleeping Sickness. (Masake, 'Sleeping Sickness Reports,' vol. 6, p. 234.)

„ 2.—Extension of the proliferated glial tissue along a septum of the spinal cord, showing the processes of the glial cells extending on to a small collapsed vessel (Masake, 'Sleeping Sickness Reports,' vol. 6, p. 234.)

„ 3.—Various stages in acute glial cell proliferation, seen abundantly in the brain tissues of the monkey, dying eight months after infection by fresh fly feeding. Lymphocytes (*l*) are also seen.

„ 4.—Ditto with branching glial cells, their processes enveloping and extending on to a wall of a small vessel.

„ 5.—Very marked glial proliferation around a small vessel. The cells are older, being much larger and their processes more numerous and extensive. A long process can be seen extending from the large branching neuroglial cell and terminating in a foot on the wall of the vessel. This represents the glial proliferation found in a monkey which died with lesions similar to human Sleeping Sickness. These drawings were made from preparations of $10\,\mu$ thickness, stained by polychrome and eosin method. The body and processes of the neuroglial cell stain pink, the nucleus purple.

„ 6.—Section of degenerated root of 30th segment of the spinal cord dourine, stained by Van Gieson's method.

All the nerve-fibres have disappeared. Preparations stained by Weigert method show that nearly all the fatty matter has been absorbed; the axis cylinders have disappeared and no regeneration has occurred. The endoneurion is highly vascular, proliferated, and increased in amount. The circular spaces corresponding to the degenerated nerves are still seen, but no nerve-structure is left. The clear space seen in the centre of the enlarged neurilemmal cell corresponds to the space formerly occupied by the axis cylinder and its coating of myelin. From its colour and high refraction, it is probably filled with fat.

Plate 2.

Fig. 1.—Drawing of the inflammatory exudation around 30th segment of the lumbo-sacral spinal cord, with attached membranes and roots, Dourine. Stained by Van Gieson's method. Magnification × 230.

R, Periphery of root with lymphocyte proliferation of very much thickened perineurion (I).

The nerve-fibres appear to be intact.

A, Two small arteries with obliteration of lumen by inflammatory thickening of their walls. Beneath there is a quantity of loose fibrous and gelatinous material with cell nuclei.

G, Posterior spinal ganglion cell with nuclear proliferation of the capsule and leucocytes.

L, Leucocytes.

Fig. 2.—Adjacent portion of the lateral column of the spinal cord, showing a leptomeningitis with great thickening of the subpial neuroglia tissue and extension of the same along the septa. Photomicrograph 6 shows the appearance presented where this was cut longitudinally. Extending from the main septa are branching septa consisting of neuroglia cells. Many of these neuroglia cells, with distinct nuclei and branching processes running round and between the nerve-fibres, can be seen. In this case the noxious agent which has caused this change has operated from the sub-arachnoid space and proceeded inwards along the lymphatics of the septa.

The drawings have been made by Miss Agnes Kelley and are faithful reproductions of the appearances presented by the specimens.

DESCRIPTION OF PHOTOMICROGRAPHS OF DOURINE.

Plate 3.

1.—Anterior horn lumbo-sacral region. The motor spinal cells show a diffuse stain, indicative of a slight coagulation necrosis. The outline of the cells is not altered much. There is a general proliferation of the glia cell nuclei and a lymphocyte infiltration, not pronounced around the capillaries and small vessels. Polychrome eosin stain. Section $10\,\mu$ thickness. Magnification ×180.

2.—Section of posterior root of 30th segment, showing degenerative atrophy of the nerve-fibres, inflammatory change in the sheath around and proliferation of the cells of the perineurion and endoneurion. Polychrome eosin stain. Sections $10\,\mu$ thickness. Magnification ×120.

3.—Section of the posterior column, showing three bands of degenerative sclerosis in the root zone. Weigert stain. Celloidin preparation.

4.—Section of posterior spinal ganglion, lumbo-sacral region. Van Gieson's stain. Showing chronic inflammatory change with atrophy and destruction of the ganglion cells. Magnification ×200.

5.—Section of lateral column 30th lumbar segment, showing septal proliferation of the glia cells without destruction of the intervening nerve-fibres. Van Gieson's stain. Magnification ×150.

6.—Longitudinal section of the subpial tissue of the 30th lumbar segment, showing neuroglia proliferation. Eisath's stain. Magnification ×850.

PLATE 4.—SLEEPING SICKNESS.

7.—Posterior spinal ganglion, case of chronic Sleeping Sickness, showing wide-spread interstitial chronic inflammation. Polychrome eosin stain. Section 10 μ thickness. Magnification ×85.

8.—Ditto. Magnification ×480. Showing the proliferation of the endothelial cells of the capsule and lymphocyte interstitial infiltration.

9.—Section of the spinal cord, chronic Sleeping Sickness, showing septal glia proliferation, partly due no doubt to degenerative atrophy of the nerve-fibres. Weigert stain. Sections 30 μ thickness. Magnification ×80.

10.—Section of a vessel with surrounding lymph space, chronic Sleeping Sickness. A meshwork of fibrils is seen, derived from the branching processes of the neuroglial cells. The neuroglia nuclei are stained, the lymphocytes are unstained. Magnification ×480.

11.—Vertical section of a perivascular space in the brain of the monkey which died of chronic Sleeping Sickness. Material kindly given by Major Leishman. Stained by polychrome eosin. Three large neuroglia cells, with branching processes, are seen, and many others in parts. Entangled in the meshwork are numerous lymphocytes. Magnification ×600.

12.—Section of monkey's brain infected with *T. Gambiense*, showing glia cell proliferation. Many of the glia cells can be seen with their processes extending on to the wall of the vessels. There is a glia cell nuclear proliferation also. Heidenhain, eosin stain. Magnification ×250.

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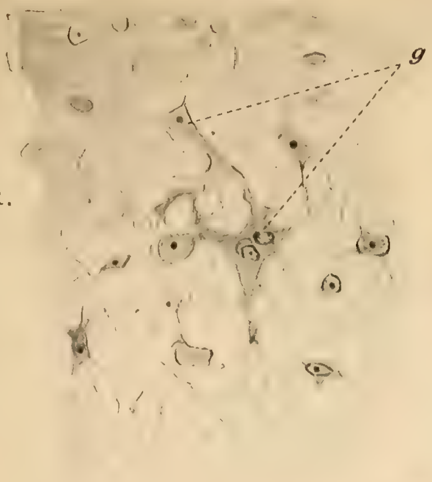

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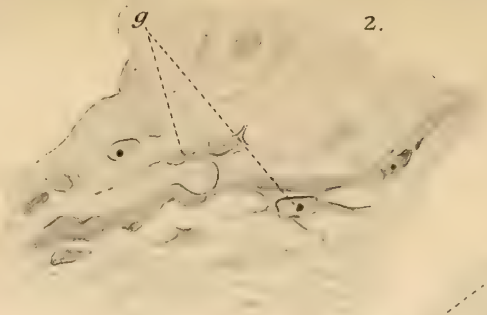

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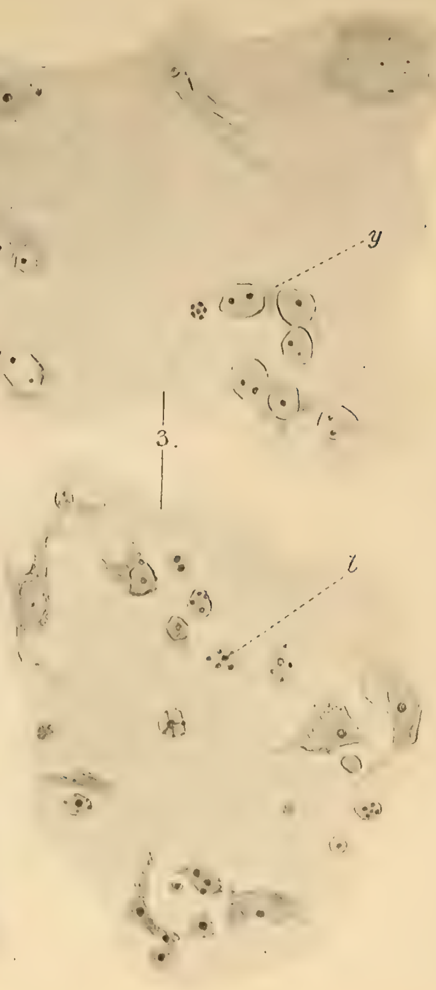

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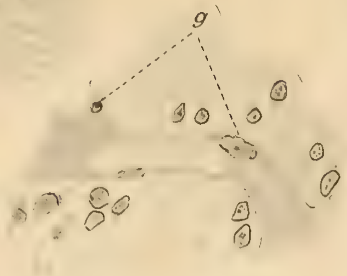

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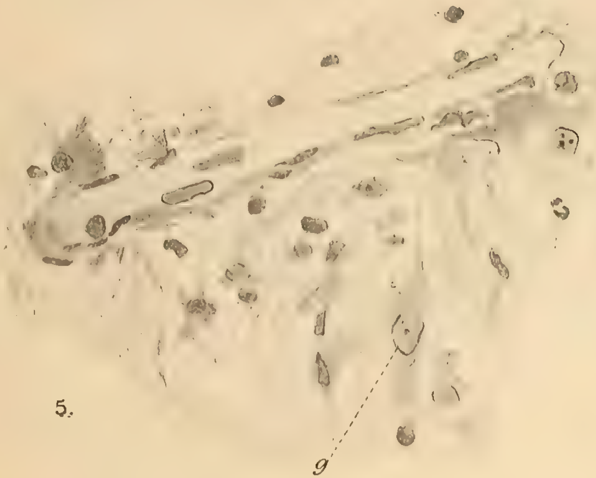

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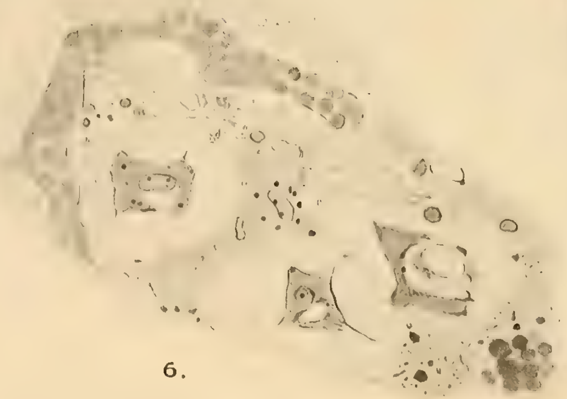
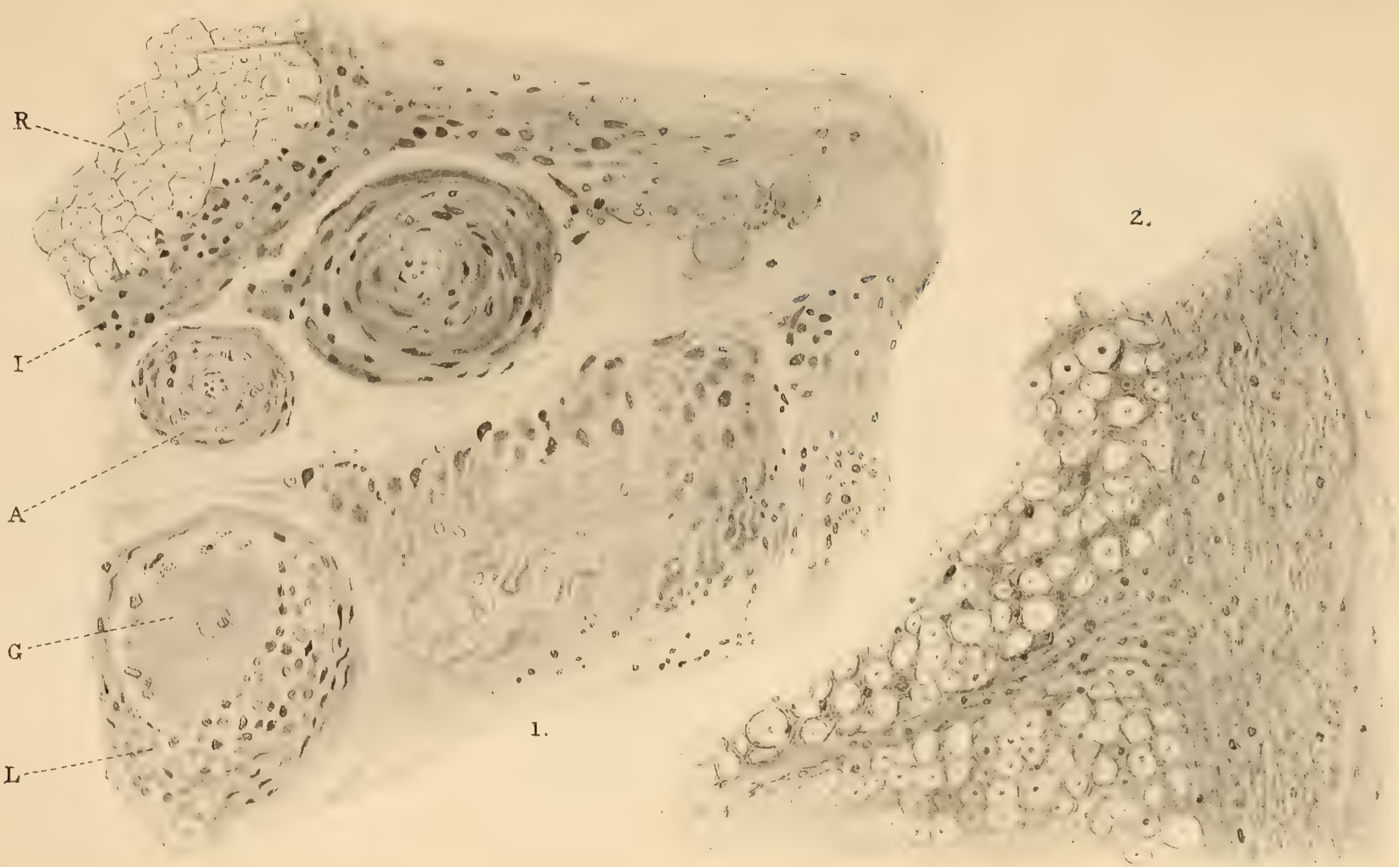


Mott. *Roy. Soc. Proc., B. vol. 78, Plate 2.*



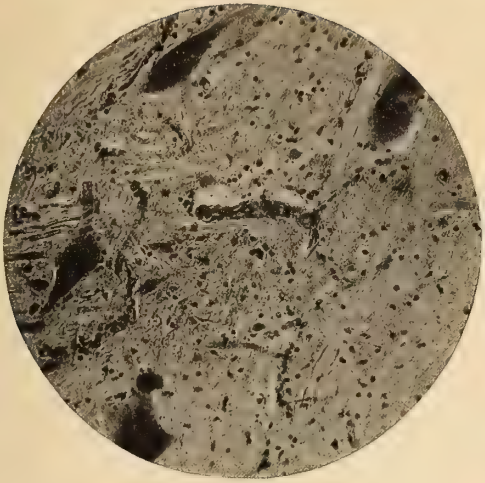


Fig. 1.

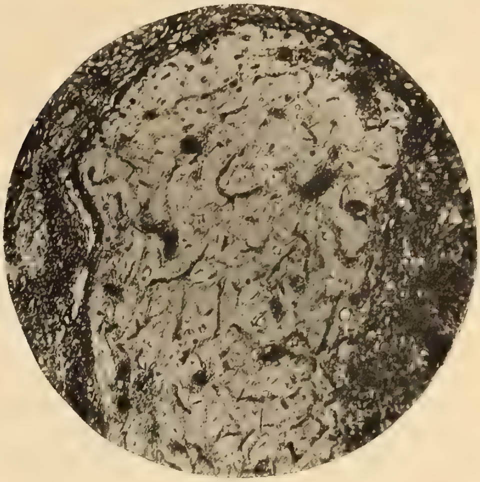


Fig. 2.

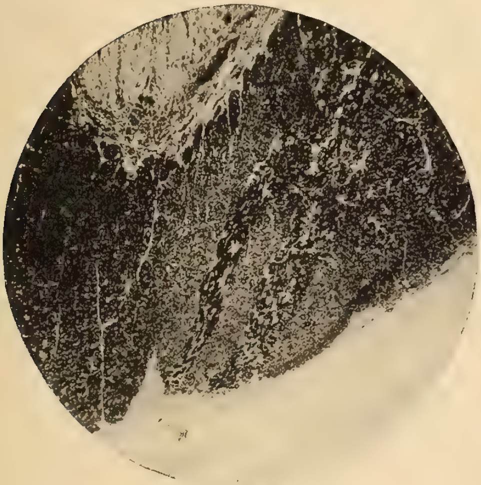


Fig. 3.

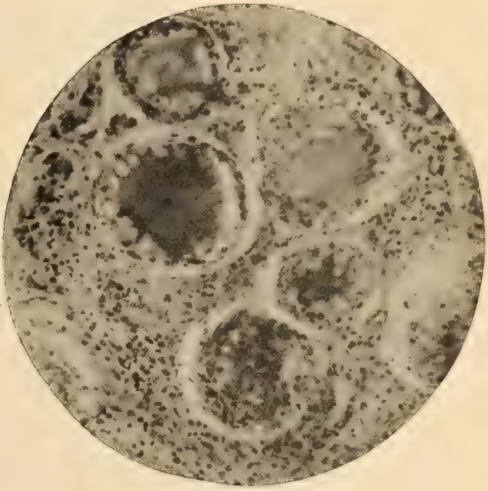


Fig. 4.

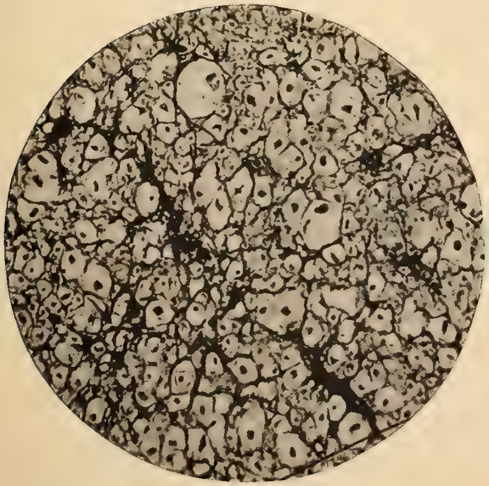


Fig. 5.

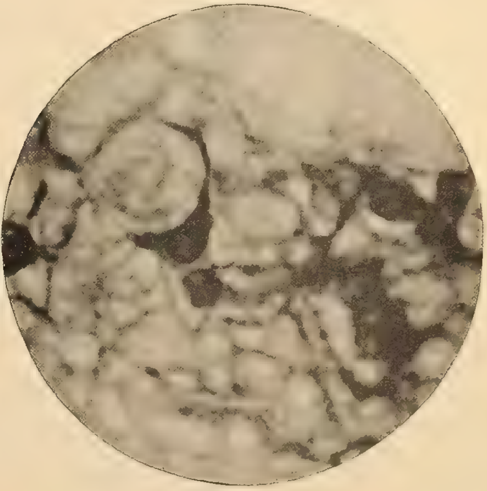


Fig. 6.

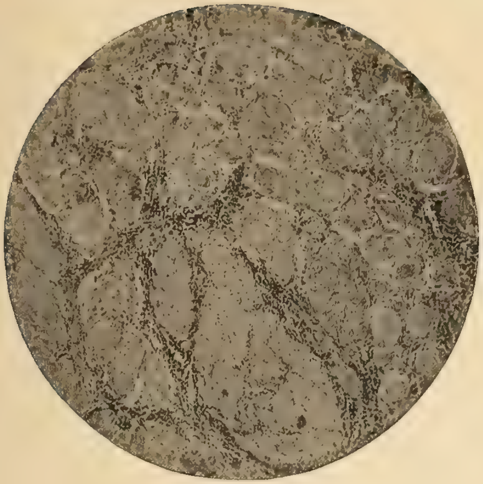


FIG. 7.

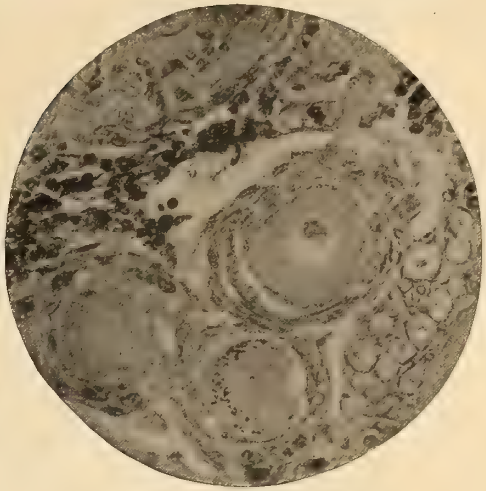


FIG. 8.

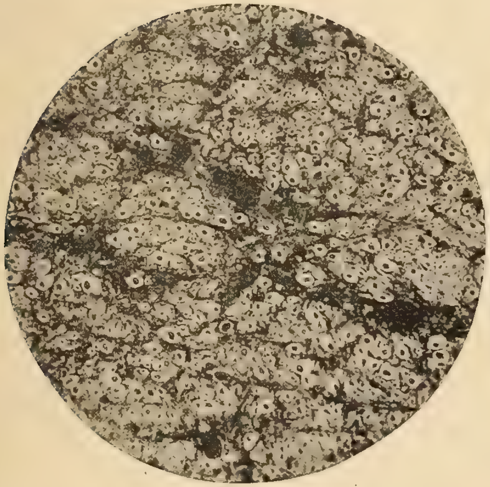


FIG. 9.

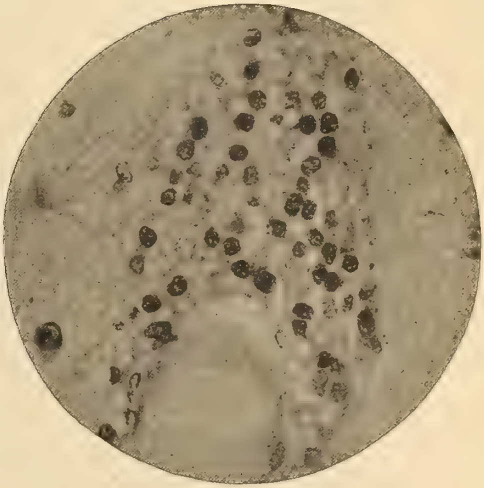


FIG. 10.

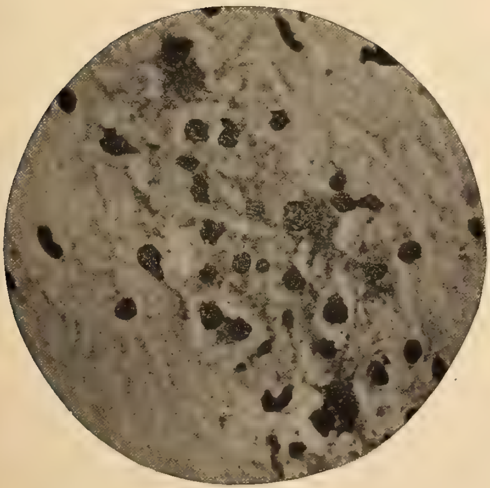


FIG. 11.

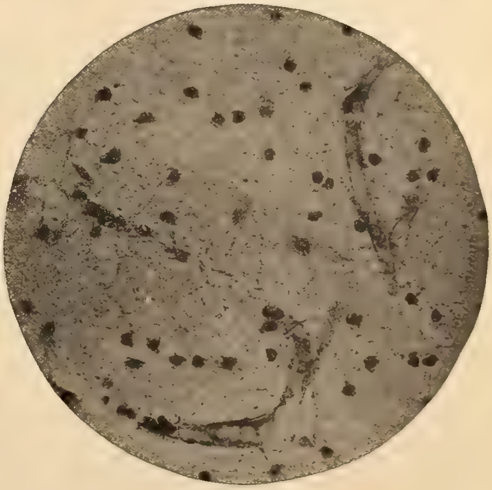


FIG. 12.

On the Physiological Action of a Recently-Discovered African Arrow Poison.

By Charles Bolton, M.D., Assistant Physician to University College Hospital.

(Communicated by Professor Sidney Martin, F.R.S. Received March 20,—
Read April 5, 1906.)

(From the Pathological Laboratory, University College, London.)

The investigation of the toxic properties of this poison was undertaken at the request of Professor Ray Lankester, to whom it was sent by Dr. David Alexander of the West African Medical Staff Corps in September, 1904.

Dr. Alexander obtained the poison from a medicine house at Ghasi, a pagan town on the Gongola River, a tributary of the Benue, in Northern Nigeria. He states that when fresh it is a semi-fluid, black, sticky substance, that it is smeared on sticks, and that when it is required for re-dipping the arrows it is scraped off and heated. It appears to be composed of, or at all events to contain, the juice of a variety of Fig, and it is stated that placenta is used in its manufacture. Dr. Alexander further states that he saw a native shot in the abdomen with a poisoned arrow, and that death resulted apparently from heart failure in about 25 minutes.

No attempt has yet been made to analyse the substance chemically, and the following remarks therefore apply entirely to its physiological action. There are many further points in relation to the action of this interesting poison which require elucidation, but as only a small amount was available for use it was thought advisable to publish the main results which have been obtained. The subject will be treated of under the following headings:—

1. Physical properties of the poison.
2. Minimum fatal dose.
3. Symptoms following its injection into animals.
4. *Post-mortem* appearances resulting from such injection.
5. Experimental investigation into the physiological action of the poison.
6. Conclusions.
7. Protocol of experiments.

1. Physical Properties of the Poison.

The poison which I received was smeared on two canes and dusted over with the powder from the inside of a gourd to preserve it. The substance was easily scraped off and found to be soft, pasty, black, somewhat granular in appearance, and possessed of a peculiar sweet smell. It dissolved quite easily in water, forming a dark greenish-brown muddy solution with a slightly acid reaction.

On centrifugalisation or filtration a greenish-brown mud, chiefly composed of starch grains, was separated, leaving a clear transparent dark brown solution. Boiling for half an hour does not affect the action of the solution.

The poison was dissolved in 0·86-per-cent. salt solution for intravenous injection into animals.

2. Fatal Dose in the Case of the Rabbit.

The solution was injected into the marginal vein of the ear in each case.

Single doses:—

gramme.				
0·15	per kilogramme weight of	animal	killed in	3 mins.
0·112	„	„	„	3 „
0·094	„	„	„	4 „
0·072	„	„	„	4 „
0·065	„	„	„	4 „
0·05	„	„	„	10 „
0·031	„	„	„	2½ hrs.
0·027	Completely recovered.			

Repeated doses:—

gramme.				
0·029	per kilogramme weight of	animal	killed in	2 hrs. (as a 2nd dose).
0·029	„	„	„	20 mins. (as a 2nd dose).
0·027	„	„	„	15 „ (as a 3rd dose).
0·019	„	„	„	20 „ (as a 4th dose).

The minimum lethal dose is therefore about 0·03 gramme per kilogramme weight, but much smaller doses (*e.g.*, 0·019) will kill if the animal has been injected on previous days.

3. Symptoms Following Injection of the Poison.

Rabbit.—There are four obvious symptoms:—

(1) Movements of the jaws, tongue, and nose; (2) dyspnœa; (3) muscular weakness; (4) restlessness and often a slight convulsion immediately before death.

With large doses of the poison injected intravenously the symptoms begin almost at once or from one or two minutes after injection. The first symptoms are licking movements and twitching of the alæ nasi, the jaws being moved as if the animal were trying to chew or swallow; sometimes it puts up its fore paw as if endeavouring to remove something from the mouth. Occasionally salivation occurs. Attacks of inspiratory dyspnœa then come on, the animal opening its mouth and stretching its neck in inspiration, and these alternate with periods of rapid shallow breathing. A general muscular weakness now prevails; the head sinks down, and the animal finally collapses and lies panting on its side. It sometimes tosses itself about in its efforts to hop, or rolls over and staggers about. Finally, a slight convulsion, in which the animal stretches itself out, occurs during the death agony.

After very small doses the only sign of poisoning may be a single attack of dyspnœa, or more prolonged and laboured breathing with movements of the jaws and tongue may occur, and the animal may subsequently recover without any weakness supervening. On auscultation of the chest air is heard to enter the lungs quite well, and the heart, as a rule, is rapid, but on one occasion for a while it was slow and irregular in rhythm.

Cat.—Similar symptoms are observed with intravenous injections as in the case of the rabbit, and they come at the same time, *e.g.*, salivation, licking, and chewing movements, the saliva being churned up into a foam, with coughing and retching, but no vomiting. Dyspnœa, inspiratory or shallow and rapid, and muscular weakness then supervene, and during a convulsion in which fæces are voided death occurs.

Guinea-pigs.—After intraperitoneal or subcutaneous injection of an amount sufficient to kill a rabbit the symptoms commence after about half an hour, and in one hour they are well marked. The animal lies on its abdomen, and on trying to move violent trembling comes on, and it staggers about. There is marked loss of power in the legs, especially the front legs, and the head sinks down as if too heavy for the muscles to support. For some time the animal can move along by means of its hind legs, but finally they become completely powerless, and it lies on its side breathing rapidly. The respirations become slower, and after a few gasps it dies. No convulsions or twitchings occur. Death takes place in about one and a half hours.

4. *Post-mortem* APPEARANCES.

The only abnormal appearances found after death are seen in the heart and large blood vessels, and these indicate that death has occurred from heart failure. The remaining organs of the body have invariably been found normal.

Heart.—This organ is flabby and dilated in both its left and right sides. Its cavities are full of blood, dark in colour in the right ventricle and bright red in the left ventricle. The large vessels are also full of blood. The heart evidently stops in diastole.

As a *post-mortem* phenomenon the left ventricle may pass into systole.

If the unfiltered or uncentrifugalised solution has been injected in large doses, the blood in all the cavities of the heart is liable to be found clotted immediately after death, the clot being black in the right cavities and red in the left cavities. The large veins are full of dark clot, and the aorta contains bright red clot. The clotting extends up to the jugular veins. The pulmonary vessels contain fluid blood, and the portal vein may contain a few clots, but here the blood as a whole is fluid.

If the solution has been centrifugalised or filtered previous to injection no clotting occurs. In all cases the blood clotted normally when removed from the body. In one case which had received four injections extending over a period of six days evidence of a gradual heart failure was found in the presence of dropsy of the mediastinum and slight bilateral hydrothorax.

Central Nervous System.—The spinal cord and bulb were stained by Nissel's method in one case, which had received several injections of the poison extending over a period of several days. The nerve cells were normal in all parts.

5. Mode of Action.

In this investigation the animals used were cats and rabbits. Ether was in all cases administered before and during the experiments.

Neither morphia nor curari was previously administered as it was thought that it would be better to rely entirely upon ether, and the results have justified this procedure. In three cases the initial blood pressure was very high, probably owing to the ether, but this did not interfere with the results, because the relative readings of the blood pressure before and after injection were only required and not the absolute readings.

The mean arterial blood pressure was taken in either the carotid or femoral artery and recorded by means of Brodie's modified kymograph. The tube used to connect the artery with the mercurial manometer was filled with a solution of $MgSO_4$ (sp. gr. 1046). The respirations were also recorded by means of a tambour, and the time marked in seconds.

Artificial respiration was employed through a tracheotomy tube in the spinal-cord experiments, the air being blown through a bottle containing ether. A secondary coil and bichromate battery were used for the stimulation experiments.

A tracing of the normal blood pressure and of the respiration was taken in all cases before the poison was injected. This substance was injected either into the femoral or jugular vein in the cats and into the marginal vein of the ear in the rabbits.

The poison was also tested with regard to its action upon the frog's heart and voluntary muscle. The solutions were made with Ringer's solution and applied with a brush. The heart beats were recorded by means of a lever in the usual way. The down-stroke represents systole.

The effects of injection of the poison upon the vascular system and respiration will first be described, after which the effects of the following procedures upon these systems under the influence of the poison will be discussed:—

Abdominal compression.
Section of vagus—
  a. Before injection of poison.
  b. After „
Excitation of vagus.
Excitation of depressor nerve.
Section of spinal cord.
Section of splanchnic nerves.
Excitation of splanchnic nerve.
Previous injection of apocodein.

Finally, the action of the poison upon the frog's heart and voluntary muscle will be considered.

Action upon the Vascular System and Respiration—Effects of the Poison alone.

Blood Pressure.—Almost immediately after the commencement of the injection, or a few seconds later, the blood pressure rises rapidly, but not suddenly. In about 10 seconds it reaches a height of about 30 to 40 mm. Hg above what it originally was. During the next few seconds the pressure curve falls a trifle, and is liable to be interrupted by sudden small depressions.

From this point there is a very slow, steady rise in the pressure, the maximum height being attained in a minute or more according to the dose. The height is now from 10 to 20 mm. Hg greater than the maximum of the first rise. From this point the pressure curve gradually falls for two minutes or more, the incline being much steeper during the last half minute.

The curve is now frequently interrupted by sudden drops in the pressure, the latter recovering itself each time less and less, till it reaches its lowest point at death. With small doses the effect is not so marked, and the final fall of pressure is very gradual, irregular small fluctuations taking place as the curve drops.

Heart.—During the first rise of pressure the rate of the heart becomes slower and the individual pulsations larger towards the end. During the slight succeeding fall the rate becomes slower still, the pulsations being larger. As the pressure curve rises the second time the rate becomes rapid, and the pulsations frequently irregular in size. As the pressure finally falls the pulsations becomes smaller, and towards the end too small to count, they are still more rapid and irregular in size and rhythm.

Respiration.—There is no effect on the respiration until the blood pressure falls towards the end, when it may become shallow.

As the heart stops, irregular respirations or large inspirations are taken. After the blood pressure has fallen to its lowest limit and there are no cardiac pulsations, inspiratory gasps may be taken at intervals for more than a minute.

It thus appears that the action of the poison is directed against the circulatory apparatus and that the effects upon the respiration are secondary. There is no evidence that respiration is directly affected; even when the voluntary muscles are paralysed the animal still breathes and gasps are taken after the heart has stopped. The blood in the left side of the heart which is found dilated is bright red, showing that asphyxia plays no part in bringing about death.

The rise of blood pressure appears to be chiefly due to vasomotor constriction, since the heart is slowed. The subsequent rapidity of the heart indicates an increased irritability, the vagus being unable to hold it in check and hence the blood pressure rises, but subsequently falls as the muscular power of the heart fails.

Effect of Abdominal Compression.

Even when the blood pressure has almost fallen to its lowest limit the heart is capable of responding to the increased amount of blood supplied to it by abdominal compression and the blood pressure rises, but if the compression is persisted in the heart fails and the blood pressure falls in spite of the compression.

This experiment illustrates the wonderful reserve power the heart possesses even when almost dead and it shows that the final fall of blood pressure is not a pure vaso-motor phenomenon, as in that case the blood pressure would be restored by abdominal compression and the heart would not suddenly fail.

There is, therefore, a direct action of the poison upon the heart muscle, the heart failure being a primary phenomenon.

Section of the Vagi.

(a) *Section before the Injection of the Poison.*—The effect of section of both vagi before the injection of the poison is to do away with the slowing of the pulse and the slight fall in the blood pressure, which occurs after the first rise in the pressure; there are in addition no depressions in the curve at this time.

The pressure gradually rises in a long curve to its maximum height and then gradually falls till death. In addition to this there is little or no increase in the pulse rate towards the end. The respiration has the usual vagus type and is unaffected by the poison, only becoming irregular as the heart stops and continuing for some time after the blood pressure has fallen.

Section of one vagus only does not produce any such alteration in the character of the curve as is produced when both vagi are divided.

Since the blood pressure rises in spite of section of both vagi, to as great an extent as it does when they are intact, the conclusion follows that the first rise of blood pressure is not due to paralysis of the vagi, and since there is no notable alteration in the pulse rate during this rise confirmatory evidence is obtained that the rise of blood pressure is chiefly due to vaso-motor constriction. The absence of slowing of the pulse during the initial rise and likewise the absence of the usual slight fall in pressure at this time when the vagi are cut, indicate that these phenomena are due to a stimulation of the vagus centre in the medulla, the effect having been abolished by section of the vagi.

I shall show later that there is no evidence whatever that the poison acts upon the central nervous system and therefore it is rendered most probable that this irritation of the vagus centre is not due to a direct action of the poison, but is merely the usual stimulation that occurs as a result of a rise of blood pressure.

(b) *Section after Injection of the Poison.*—Section of one vagus brings about a rise in the blood pressure and acceleration of the pulse at an early period whilst the blood pressure is rising, and section of the vagus on the other side does not increase this rise at a slightly later period. It is only at an early period of the injection whilst the blood pressure is rising that section of the vagus causes a further rise, section at a late period, and especially when the blood pressure is falling, bringing about no change whatever in the blood pressure curve or the rate of the heart. In one case section of the right vagus 54 minutes after injection followed by section of the left vagus 19 minutes later produced no rise at all. The section, therefore, to produce any rise in blood pressure must be done at an early period after the injection.

These results show that the vagus is certainly exerting its tonic effect upon the heart during the early stages of the poisoning and that, therefore, the first rise of blood pressure is not due to vagus paralysis but is probably of peripheral origin. Later on the vagus has lost control over the heart and its section produces no effect.

The fall of blood pressure which occurs in the later stage is, therefore, due to gradually increasing weakness of the heart muscle and not to vagus irritation.

Excitation of the Vagus.

Two effects are to be noticed:—

1. In the early stages of the rise of blood pressure excitation produces a fall of blood pressure with slowing of the heart, but the effect is usually much less than in the case of the normal animal. Before the blood pressure reaches its maximum, excitation of the vagus produces little or no effect whatever upon the blood pressure and during the final fall excitation produces no effect.

2. Instead of the pressure falling at once as it does in the normal animal on stimulation, there may be a latent period of a greater a less length which becomes longer towards the end as the vagus ceases to produce any effect. Finally prolonged stimulation produces no effect at all.

These results of excitation of the vagus point to the same conclusion as was deduced from the results of section of that nerve after injection of the poison—namely, that the vagus is exercising its tonic influence upon the heart, though to a less extent than normal, in the early periods, but that it gradually ceases to exert this tonic influence owing to increased irritability of the heart muscle, the blood pressure now rising to its maximum, and that subsequently it has lost all control over the heart.

It is more likely that the vagus is unable to hold the heart in check, owing to increased irritability of the heart muscle than that the vagus is paralysed, because prolonged stimulation of the nerve may sometimes produce an effect; if the vagus was paralysed this would not happen.

In addition to this, stimulation of the vagus may produce an effect upon the respiration, although the blood pressure is unaffected, showing that the nerve is not totally disabled, and it is unlikely that the nerve ends in the heart would be paralysed whilst those in the walls of the bronchi were unaffected.

Excitation of the Depressor Nerve.

Rabbits are used for these experiments as the depressor nerve can be isolated in that animal. Excitation of the central end of the depressor nerve at all stages of the poisoning brings about a fall of blood pressure. As the blood pressure is falling towards the end of life the effect of excitation of the depressor is liable to be less marked.

In the early stages of poisoning the depression in blood pressure may not be so marked with the same strength of stimulus as it was before the poison was injected. This result might be expected to occur as the irritability of the heart is increased. The fact that the depressor nerve will produce an effect at all stages of the poisoning shows that the central and peripheral vaso-motor nervous mechanisms are acting normally and that there is no central or peripheral nervous paralysis.

Section of the Spinal Cord.

It was found that after section of the spinal cord in the upper cervical region, the blood pressure still rose on injection of the poison to the same extent relatively as it did in the experiments with the spinal cord intact. The vaso-constriction, therefore, is obviously not of central origin.

This rise of pressure is, however, due to constriction of the peripheral vessels chiefly, because the heart by stimulation can only cause an insignificant rise of arterial pressure when the peripheral resistance is greatly diminished, as when the spinal cord is divided.

Section of the Splanchnic Nerves.

The result of section of the splanchnics is the same as that obtained in the case of section of the spinal cord. The pressure goes up as much relatively as when the splanchnics are undivided. This is confirmatory evidence of the observation that the vaso-motor action is not central.

Excitation of Splanchnic Nerve.

Stimulation of the splanchnic nerve even at a late stage of the poisoning, when the blood pressure is following, still causes a rise in the blood pressure, thus showing that the peripheral ends of the vaso-motor nerves are not paralysed.

Previous Injection of Apocodein.

Apocodein, as is well known, brings about a fall of arterial blood pressure by paralysing the terminations of the vaso-motor nerves.

After the injection of apocodein the central end of the sciatic nerve was stimulated to make certain that no rise of arterial blood pressure occurred, thus showing that the apocodein had acted upon the nerve endings. Under these circumstances, when the blood pressure had fallen as the result of the injection of apocodein, the subsequent injection of the poison still produced a marked rise in arterial blood pressure, both when the vagi were divided and when they were intact.

This result proves that the rise of blood pressure is due to a direct stimulation of the muscular coats of the arterioles. There is no evidence to show that the vaso-motor nerves are in the least affected by the poison.

Action upon the Frog's Heart.

When applied to the frog's heart the effect of the poison is to gradually diminish the size of the beats till the ventricle is brought to a stand-still in diastole, the auricle continuing to beat for a little while longer and then also stopping in diastole.

Sometimes a block is seen at the auriculo-ventricular junction, only every second beat crossing over, and occasionally irregular peristaltic waves, commencing at the base, pass over the ventricle. The ventricle may take on an independent rhythm. The rate of the heart is not altered until just before it stops, when slight slowing is noticed.

Application of atropine to the heart in arrest fails to restore its action, thus showing that the standstill is due to a direct action on the muscular tissue of the heart and not to stimulation of the terminations of the vagus. The same result is seen if the atropine is applied before the poison.

If the heart be thoroughly washed with Ringer's solution, its action may be restored, if it has not completely come to a standstill. If the sinus be stimulated with an electric current when the heart has stopped, the action may be temporarily restored.

Action upon the Voluntary Muscle of the Frog.

The poison was applied to the muscle with a brush, and at each application the muscle shortened very slightly. The nerve was stimulated with an induced current and the poison continuously applied until the muscle failed to respond to the stimulus. The muscle itself was now stimulated, and entirely failed to respond to any strength of current.

This proves that the muscular tissue itself is paralysed by the poison. The experiment, of course, does not prove that the poison does not at the same time act upon the nerve ends, but the object of this experiment was to prove whether or not the muscular tissue itself was paralysed.

6. CONCLUSIONS.

The poison selects muscular tissue for its action and there is no evidence whatever that it produces any action upon the central or peripheral nervous system. It paralyses the voluntary muscles and brings about death by a direct action upon the muscular tissue of the heart.

The first effect of the poison is to stimulate directly the muscular coats of the arterioles and thus to cause a rise of arterial pressure.

The raised arterial pressure excites the vagus centre in the medulla, and brings about a slowing of the rhythm of the heart, together with a slight fall in the arterial blood pressure. The irritability of the heart muscle is greatly increased and soon the vagus fails to hold it in check, with the result that the rhythm of the heart is accelerated and the blood pressure therefore rises to a higher level as the inhibition of the vagus is overcome. It is, however, probable that the chief factor in maintaining the high blood pressure is the constriction of the peripheral vessels.

The rhythm of the heart becomes more rapid and irregularity appears, the heart finally passing into delirium cordis and ceasing in diastole.

As the heart fails, the blood pressure falls more or less rapidly, and the fall is interrupted by rises in which the heart temporarily recovers itself. The final fall of blood pressure is due entirely to heart failure, the muscular coats of the arteries being capable of response to stimulation till the very end. Any effect upon the respiration is secondary to the effect upon the vascular system. The final convulsion is also secondary and not due to any direct action of the poison.

In the case of the frog the action of the poison is also directed towards the muscular tissue of the heart and the voluntary muscles leading to a gradual paralysis. With regard to the action on the heart, all that can be said is that the poison acts atonically on the muscular tissue like lactic acid and potash salts, and that its conductivity is diminished.

In conclusion, my best thanks are due to Professor Sidney Martin, F.R.S., for his kindness in giving me this work to do, and also in allowing me the use of his laboratory and apparatus.

7. PROTOCOL OF EXPERIMENTS.

I. *Experiments to Determine Symptoms, Fatal Dose, and* Post-Mortem *Lesions.*

Experiment 1.—Rabbit, weight 2125 grammes. Injection of 1/5 gramme of poison dissolved in 2 c.c. salt solution (= 0·094 gramme per kilogramme). into the marginal vein of left ear. The solution was unfiltered. Almost immediately the animal began to open its mouth during inspiration, which was slow and laboured, the dyspnœa being inspiratory. The alæ nasi twitched. The animal tried to hop, but staggered and swayed. The limbs then became paralysed, the head dropped, the animal finally sank on its side and during a convulsion in which the body was stretched out rigidly it died. Death occurred in four minutes.

Post-mortem.—The heart was dilated. Both auricles and ventricles were full of clotted blood, on the right side black, on the left side bright red. The aorta also contained red blood clot. The portal vein contained a few dark blood clots, but the blood in it was mostly fluid. The venæ cavæ and jugular veins contained dark clot. The blood in the pulmonary veins was fluid.

Experiment 2.—Rabbit, weight 890 grammes. Intravenous injection of 1/10 gramme of filtered solution of poison (=0·112 gramme per kilogramme).

Immediately after the injection the animal commenced licking movements with the tongue and chewing movements. It pawed its mouth as if trying to remove something. Inspiratory dyspnœa was not such a marked feature as in the former case. Muscular weakness supervened and during a convulsion the animal died in three minutes after the injection. The hind legs were stretched out, the spine rigid and the head retracted.

Post-mortem immediately after death. The heart was dilated. No clots were found in any of the vessels. The blood on the left side of the heart was bright red, and on the right side dark in colour.

Experiment 3.—Rabbit, weight 1270 grammes. Intravenous injection of 1/5 gramme of the unfiltered solution (= 0·15 gramme per kilogramme). Chewing and licking movements began at once and then inspiratory dyspnœa, but this was not such a marked symptom as when smaller doses were used. The animal collapsed on its belly and a convulsion occurred, the head being retracted, the spine opisthotonic, and the hind legs extended. Death occurred in three minutes.

Post-mortem immediately after death. The changes were exactly the same as in the case of Experiment 1, and the blood clots were found in the same situations.

Experiment 4.—Rabbit, weight 1150 grammes. Intravenous injection of 1/12 gramme of the filtered solution (=0·072 gramme per kilogramme). In one minute the chewing and licking movements began and the animal threw its head backwards and forwards. Severe inspiratory dyspnœa occurred. General weakness supervened and after a convulsion death occured four minutes after injection of poison.

Post-mortem.—The heart had evidently stopped in diastole. No clots in any part of the vascular system were seen.

Experiment 5.—Rabbit, weight 1150 grammes. Intravenous injection of 3/40 gramme of filtered solution (= 0·065 gramme per kilogramme). The olution was *boiled* for half an hour and then allowed to cool before injection.

Death occurred in four minutes, the same symptoms occurring as described above. The final convulsion was practically absent.

Post-mortem.—The heart was dilated. No clots were found in any portion of the vascular system.

Experiment 6.—Rabbit, weight 1000 grammes. Intravenous injection of 1/20 gramme filtered solution (= 0·05 gramme per kilogramme). No symptoms occurred for five minutes and then the licking and chewing movements commenced, the animal appearing to try and remove some object from its mouth. Inspiratory dyspnœa occurred and finally muscular weakness. Immediately before death a convulsion occurred. Death took place 10 minutes after the injection.

Post-mortem.—The heart was in diastole. No clots were seen anywhere and the left side of the heart contained bright red blood as usual.

Experiment 7.—Rabbit, weight 1050 grammes. Intravenous injection of 1/30 gramme of the filtered solution (= 0·031 gramme per kilogramme). The symptoms began in exactly the same way as in all the previous rabbits. Inspiratory dyspnœa came on in an attack which lasted three minutes and was succeeded by a period of rapid shallow breathing of 160 per minute. In seven minutes an attack of inspiratory dyspnœa occurred which lasted a few minutes and this was again succeeded by a period of rapid breathing.

After one hour general weakness began to come on and the head drooped. The animal could hop and was not paralysed, but certainly weak. Respirations 160.

Two hours after the injection the animal was lying on its belly with the head thrown back and breathing rapidly. It could not hop, but rolled over on attempting to do so. It was apparently paralysed in the legs, but could still crawl on the belly.

The weakness became more profound and death occurred in two and a half hours after the injection, a slight convulsion, the whole body being stretched out and the head retracted, occurring just at death.

Post-mortem.—The heart was dilated and no clots were seen anywhere in the blood vessels. The blood in the left ventricle was bright red.

Experiment 8.—Rabbit, weight 1800 grammes. Subcutaneous injection of 1/20 gramme of the filtered solution (= 0·027 gramme per kilogramme). The animal developed no symptoms, and five days later it was quite normal.

On the fifth day after the injection, another injection of 1/20 gramme was given intravenously. Movements of the mouth and tongue occurred and slow inspiratory dyspnœa, the head being extended in inspiration and the mouth opened. In 10 minutes the inspiratory dyspnœa disappeared and the breathing became rapid.

The next day the animal seemed rather weak but otherwise normal. On this day another dose of 1/20 gramme was given intravenously. The same symptoms supervened and rapidly a general weakness appeared. Inspiratory dyspnœa was succeeded by rapid breathing and the animal died a quarter of an hour after the injection. Two spasms and fibrillary twitchings of the muscles occurred before death.

Post-mortem.—The heart was dilated, the cavity of the left ventricle being globular. The urine in the bladder contained albumen. The medulla and cervical and lumbar enlargements of the spinal cord were hardened, cut and stained by Nissl's method. No abnormal cells were to be seen.

Experiment 9.—Rabbit, weight 2125 grammes. Intravenous injection of 1/40 gramme of the filtered solution (= 0·011 gramme per kilogramme). In a quarter of an hour the animal was seized with an attack of inspiratory dyspnœa, but soon recovered from this and showed no further symptoms. The next day 1/16 gramme (= 0·029 gramme per kilogramme) was injected into a vein. In three minutes the movements of the mouth began and inspiratory dyspnœa also. Respiration, 30 per minute. Pulse 292 per minute. The heart sounds were normal and on auscultation of the bases of the lungs, air was heard to enter freely. Attacks of slow inspiratory dyspnœa alternated with attacks of rapid breathing. The animal died in two and a half hours after the injection.

Post-mortem.—Heart dilated. No other lesion.

Experiment 10.—Rabbit, weight 1350 grammes. Intravenous injection of 1/30 gramme of the filtered solution (= 0·024 gramme per kilogramme). The movements of the tongue and mouth began in one and a half minutes, and one minute later inspiratory dyspnœa commenced. Several attacks of inspiratory dyspnœa alternated with attacks of rapid breathing. No muscular weakness was to be seen. In three hours from the injection the animal had practically recovered. The next day 1/25 gramme (= 0·029 gramme per kilogramme) was injected intravenously. The same symptoms ensued and death occurred in 20 minutes after a few convulsive movements.

Post-mortem.—Heart dilated. No other lesion.

Experiment 11.—Rabbit, weight 1700 grammes. Intravenous injection of 1/30 gramme of the filtered solution (= 0·019 gramme per kilogramme). In three minutes inspiratory dyspnœa commenced. On auscultation air was found to enter the lungs all right. The heart was very irregular and 74 per minute in frequency. The irregularity seemed to affect both force and rhythm, the beats tended to occur in groups of threes and fives.

In about eight minutes the heart became regular, and 148 per minute in frequency; the respirations were 142 per minute, and the inspiratory dyspnœa ceased. After this the animal recovered. Three days later a second injection of 1/30 gramme was given. The same symptoms occurred, together with some muscular weakness.

The following day a third dose of 1/30 gramme was given. The symptoms were more severe than before. Half an hour after the injection the animal was lying collapsed and could not stand. Attacks of inspiratory dyspnœa occurred. The next day the animal had recovered, and a fourth injection of 1/30 gramme was given. The same symptoms occurred, and after a convulsion the animal died in 20 minutes.

Post-mortem.—The heart was dilated as in the other animals. The chest contained a little fluid on both sides, and there was œdema of the mediastinum. No clots could be found in the heart and vessels.

Experiment 12.—Cat, weight 2450 grammes. Intravenous injection of 1/5 gramme of the unfiltered solution (= 0·081 gramme per kilogramme). Very rapid breathing came on almost immediately, then the animal had a violent convulsion in which fæces were passed, and died at once (three minutes after the injection).

Post-mortem, immediately after death. The heart was distended with blood clot, which was bright red on the left side and extended into the aorta, and on the right side was dark in colour and extended into the venæ cavæ. There was no clot in the pulmonary vessels or portal vein.

Experiment 13.—Cat, weight 2120 grammes. Intravenous injection of 1/10 gramme of the filtered solution (= 0·047 gramme per kilogramme).

In two minutes after the injection the animal began to salivate, and licking and chewing movements commenced. No vomiting occurred, but a sort of retching and coughing, as if the animal were trying to remove some object from the throat. Inspiratory dyspnœa then commenced, and was succeeded by rapid breathing, the animal lying on its stomach with the head bent down. Then, after a violent convulsion, during which fæces were passed and the animal threw itself about, it died in six minutes after the injection.

Post-mortem.—The heart was dilated in both its cavities. No clotting of the blood in the heart or any of the vessels was seen.

Experiment 14.—Guinea-pig, weight 410 grammes. Intraperitoneal injection of 1/20 gramme (= 0·12 gramme per kilogramme). No symptoms appeared for about half an hour, and in one hour they were well marked. The animal lay on its belly, and when it attempted to run violent trembling came on and it staggered about. There was marked loss of power in the legs, especially the front ones, and the head sank down as if too heavy for the body. For some time the animal could run along, using chiefly the hind legs, but the weakness increased, and finally it could not move at all. If rolled on to one side it could hardly right itself. Finally it sank on to its side and, after a few gasps, died one and a half hours after the injection. The respirations were at first accelerated, but just before death they were slow. No convulsions or twitchings whatever occurred, and no inspiratory dyspnœa or chewing movements.

Post-mortem.—The heart was in diastole, and the left auricle in a state of fibrillary contractions. No clotting.

Experiment 15.—Guinea-pig, weight 410 grammes. Subcutaneous injection of 1/20 gramme of the poison (= 0·12 gramme per kilogramme). The symptoms began about the same time as those of the former pig. The respirations were accelerated, and there was marked shivering and trembling. The same weakness occurred, being most marked at first in the fore legs and head. After passing through a stage of almost complete paralysis the animal died one hour and ten minutes after the injection.

Post-mortem.—The heart went on beating with irregular and spasmodic contractions for ten minutes after death. No clotting.

II. *Blood-Pressure Experiments.*

In all the following blood-pressure experiments the poison was dissolved in salt solution, which was filtered or centrifugalised before injection. In the stimulation experiments the strength of the current was measured by the distance in centimetres of the secondary from the primary coil.

Poison Only.

Experiment 1.—Cat, weight 2610 grammes. Poison 1/8 gramme (= 0·047 gramme per kilogramme).

Blood Pressure.—The curve is naturally divided into two parts, corresponding to a sharp rise in the pressure with a succeeding fall, and a second long and slow rise to a maximum with a succeeding fall till death.

The pressure curve was unaltered for 12½ seconds after the injection, and the first rise then occurred. The pressure rose somewhat abruptly from 166 to 178 mm. Hg to 200 to 210 mm. Hg in 10 seconds, and during the next six seconds it fell to 184 mm. Hg. The second rise of pressure was more gradual, and attained its maximum point of 224 mm. Hg in the succeeding 73 seconds, the pressure during this time having risen 40 mm. Hg. From this point the curve gradually fell for 54 seconds, and then more rapidly for the last 38 seconds of life.

During the final fall there were three sudden descents of about 80 mm. Hg in the curve, obviously due to heart failure. The heart recovered itself after the first two falls, the pressure shooting up 60 and 40 mm. Hg respectively. The last fall was practically to the lowest point of the curve, and the heart only recovered itself by 10 mm. Hg before ceasing as the pressure fell to its lowest point of 20 mm. Hg above zero. It will be seen that the respiratory rhythm of the blood pressure becomes lost towards the end.

Heart Beats.—The rate of the heart was 213 per minute before injection. Until the pressure rose there was no alteration in the rate. During the first rise of pressure the heart beats became rather slower as the summit of the curve was reached; the number of beats during the 10 seconds of the first rise was 33, making a rate of 198 per minute. The beats also became rather larger. During the first fall of pressure, which lasted six seconds, 16 pulsations occurred, making a rate of 160 beats per minute, the pulsations also became larger.

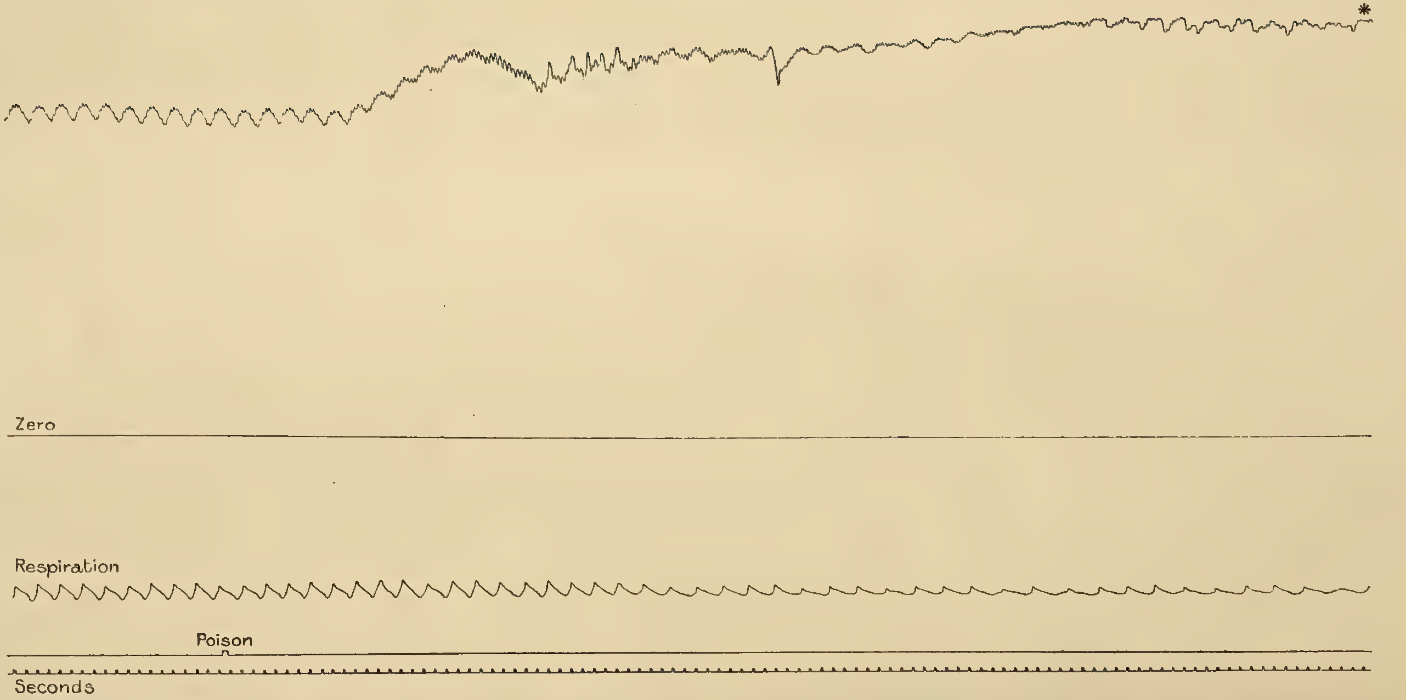
During the second rise of pressure the heart became gradually more and more rapid as the summit of the curve was reached, the rate at this point being 216 per minute. At the first part of the rise the pulsations were very irregular in size; towards the end they became much smaller as the rapidity increased, and they were also irregular. During the second and final fall of pressure the pulsations were very rapid and small, and towards the end were uncountable, the curve being practically a straight line.

Respirations.—The respiration was unaffected till the blood pressure fell to its lowest level, when five large inspirations were taken, inspiratory gasps following at intervals for the next 104 seconds, the blood-pressure curve meanwhile forming a straight line at about 20 mm. Hg above zero.

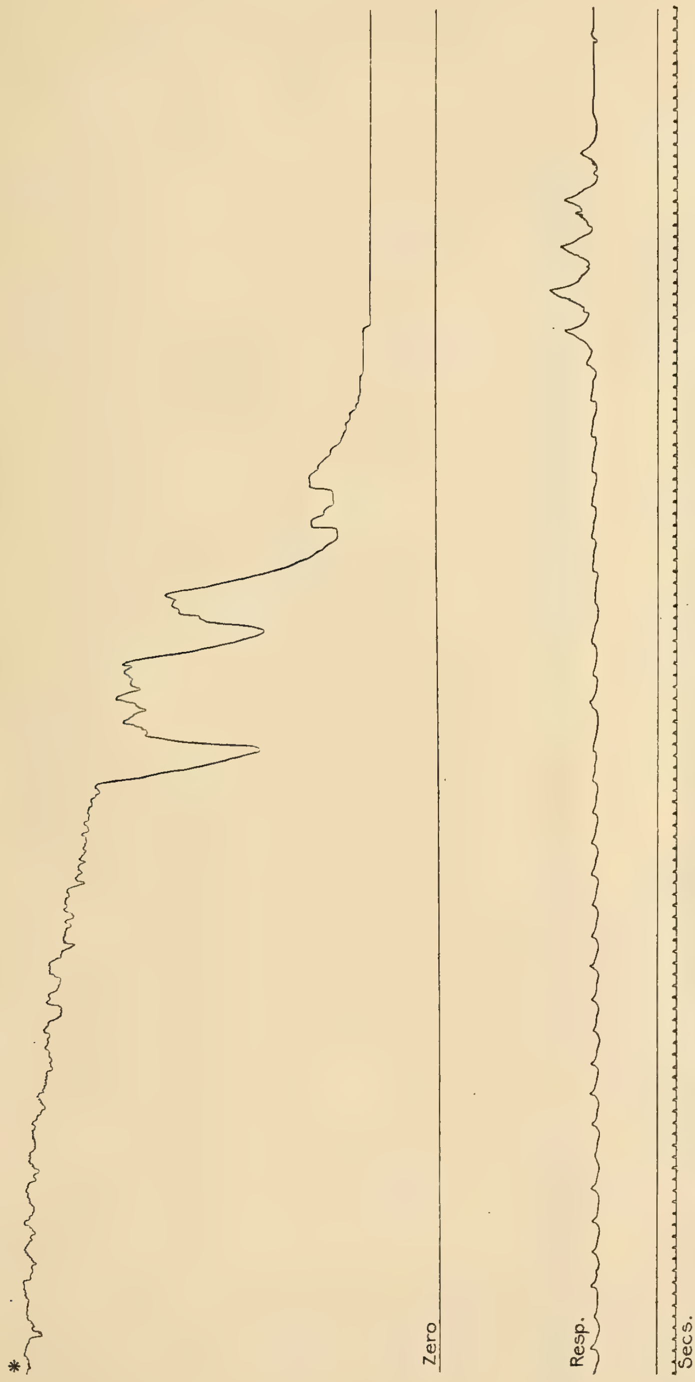
Experiment 2.—Cat, weight 1950 grammes. Poison 1/12 gramme (= 0·042 gramme per kilogramme).

Blood Pressure.—The first rise was very well marked, and commenced immediately the injection was given, and the subsequent fall also, but the second rise was not seen, the pressure, after maintaining itself for a little while, gradually dropping to its lowest point.

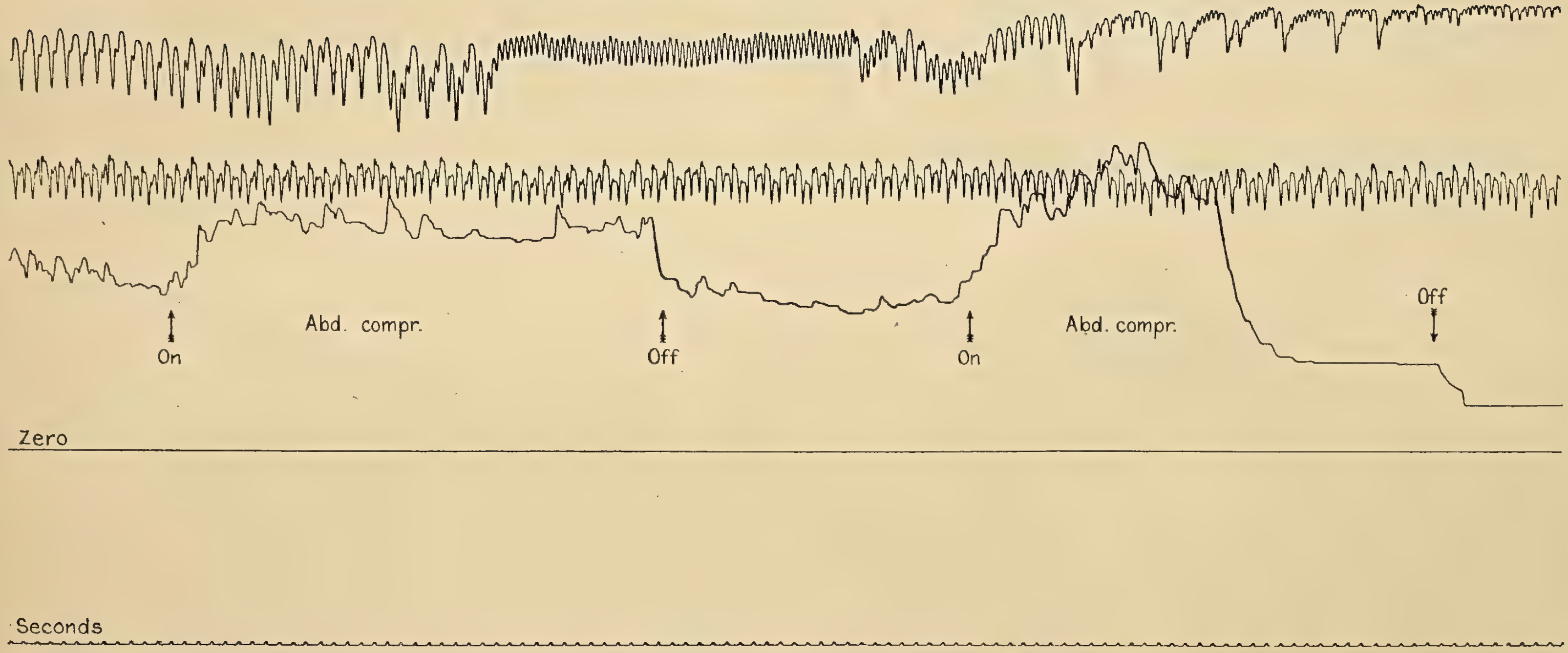
The pressure before injection was 120 to 126 mm. Hg, and the first rise reached its maximum point of 178 to 184 mm. Hg in 40 seconds. It was 154 to 164 mm. Hg 80 seconds later, and from this point it fell gradually to its lowest point of 20 mm. Hg above the zero line. There were no sudden falls of pressure as the curve was descending. The second rise in pressure, as I have already mentioned, is due to some extent to the heart, and it did not occur in this case, probably because that organ was unable to maintain the pressure. The cat was not a strong animal, and it breathed somewhat irregularly the whole time. The respiratory rhythm of the pressure curve becomes lost towards the end.



Experiment 1.—This tracing shows the effects of injection of the poison alone. The point of injection of the poison is marked on the line immediately above the tracing of the time, which is marked in seconds. An initial rise of blood pressure is seen followed by



a slight fall associated with a slower rate and increased size of the heart beats. The pressure then slowly rises to a maximum and falls till death occurs. The respiration stopped after the heart, and gasps were taken for 104 seconds subsequently.



Experiment 3.—This tracing illustrates the effect of abdominal compression as the blood pressure is falling. The drum had gone round three times, and when the abdomen was compressed the blood pressure was reaching its lowest point. On the second occasion it is seen that the heart suddenly failed during the compression.

Heart Beats.—At the end of the first rise of pressure the heart became a little slower and the beats slightly larger. At the end of the first fall the rate of the pulsations had dropped from 195 per minute to 126 per minute, and were decidedly larger. After this point till death the pulsations became gradually smaller and the rate more frequent, the latter being 246 when they could be last counted, about 100 seconds before the pressure reached its lowest point.

Respiration.—Before the injection was given the respirations were a little irregular, and they were quite unaltered by the injection. They became a trifle shallow during the last minute of life. As the heart stopped about 10 small irregular respirations occurred, and a minute later three small inspiratory gasps at intervals of 10 seconds were taken.

Compression of Abdomen.

Experiment 3.—Cat, weight 3220 grammes. Poison 1/10 gramme (= 0·031 gramme per kilogramme). A smaller dose of the poison was given so as to produce a slow heart failure. The animal lived for about half an hour. The kymograph drum was allowed to go round twice, and it had commenced its third revolution when the blood pressure, which had been gradually falling, reached 60 mm. Hg. The abdomen was compressed, and the blood pressure immediately went up to 95 mm. Hg; the compression was kept up for 37 seconds, and as soon as it was relaxed the blood pressure immediately fell to 60 mm. Hg again.

The abdomen was again compressed for 35 seconds. The blood pressure rose to a maximum of 120 mm. Hg, and when the compression had been maintained for 18 seconds the blood pressure suddenly fell straight down to 34 mm. Hg and the heart stopped. When the compression was relaxed there was a further fall to 20 mm. Hg above zero. The heart had failed before the extra work that was presented to it by increasing the flow of blood into its right cavities during diastole.

Section and Excitation of the Vagi.

Experiment 4.—Cat, weight 2750 grammes. Both vagi divided before injection. Poison 1/10 gramme (= 0·036 gramme per kilogramme), the dose being repeated.

Blood Pressure.—Immediately the injection had been given the blood pressure began to rise, and during the first six minutes after the injection it rose from 162 to 180 mm. Hg to a maximum of 194 to 210 mm. Hg. After this it gradually fell to 184 to 162 mm. Hg, and now a second injection of 1/10 gramme of the poison was administered. After the second injection the pressure rose to 169 to 170 mm. Hg in 33 seconds and then fell rapidly to 30 mm. Hg above zero owing to heart failure.

This pressure curve cannot be divided into two parts as described above when the vagi are intact. The pressure gradually rose in a long single curve to its highest point, and from this point it gradually fell.

Respiration.—The respirations were not affected till the blood pressure finally fell, except that they became slightly shallower. Large inspiratory gasps were taken for three minutes after the blood pressure had fallen to its lowest limit.

Experiment 5.—Cat, weight 3220 grammes. Both vagi divided before injection. Poison 1/10 gramme (= 0·034 gramme per kilogramme).

Blood Pressure.—The blood pressure began to rise six seconds after the injection of the poison, and attained its maximum height of 216 mm. Hg in 86 seconds, having risen from 174 mm. Hg. After this there was a steady fall till the heart stopped in 146 seconds.

Heart Beats.—Immediately before injection the rate of the heart was 258 per minute, and when the blood pressure had risen to its maximum point the rate was unaltered. Before the final fall it had increased to 261 per minute. There was no fall in the blood pressure during the early stage of poisoning, and the pulse rate did not notably increase towards the end of life.

Respirations.—The respirations were not affected until the blood pressure fell, when they became irregular as the heart stopped, inspiratory gasps continuing at intervals for about three minutes after the blood pressure had reached its lowest point.

Excitation of Vagus.—The left vagus was stimulated, the strength of current being 10 (secondary coil at 10 cm. distance). The stimulation was continued for 10 seconds. Before stimulation the blood pressure was 216 mm. Hg, and at the end of stimulation it was 210 mm. Hg; for the next six seconds it gradually fell to 190 mm. Hg, and then rose almost to its former height (210 mm. Hg). The rate of the heart fell by about eight beats per minute.

The vagus was again stimulated 50 seconds later, the strength of the current being increased to six and the time to 12 seconds. This time there was no obvious effect produced upon the blood pressure. Before the poison was administered a stimulus of strength 12 applied to the left vagus produced an immediate fall in the blood pressure of over 30 mm. Hg.

Experiment 6.—Cat, weight 2720 grammes. Right vagus divided before injection. Poison 1/15 gramme (= 0·024 gramme per kilogramme).

Blood Pressure.—The pressure curve began to rise 13 seconds after the commencement of the injection, and in 26 seconds it had risen from 160 mm. Hg to 182 mm. Hg. There then occurred a slight fall in the pressure to 178 mm. Hg. After 220 seconds the pressure had risen to 200 mm. Hg, its maximum point, and then succeeded a rapid fall to 20 mm. Hg as the animal died.

Pulse Rate.—Before the injection the rate of the heart was 228 per minute after the preliminary rise of pressure, and when it was falling the rate fell to 204 per minute. It again increased, and 120 seconds after the injection, which was the last point at which it could be counted, it had reached 240 per minute.

Respiration.—The respiration was unaffected and became spasmodic as the heart stopped. After the blood pressure had fallen to its lowest point a series of very deep inspiratory gasps occurred, which gradually died away in four minutes.

Stimulation of the Right Vagus.—Before the poison was administered stimulation of the vagus (strength 12) produced a fall in the blood pressure of over 30 mm. Hg. The vagus was stimulated 93 seconds after the injection was given (strength 12) for 15 seconds. After the stimulation had ceased a hardly appreciable fall in the blood pressure occurred. The vagus was stimulated 40 seconds later (strength 10) for 17 seconds, and during the stimulation the pressure rose somewhat higher. When the pressure had commenced to fall rapidly, a third stimulation for five seconds (strength 10) produced no effect on the pressure curve.

Experiment 7.—Cat, weight 2500 grammes. Poison 1/15 gramme (= 0·026 gramme per kilogramme).

Blood Pressure.—Ninety seconds after the injection the blood pressure had risen from 184 mm. Hg to 216 mm. Hg. The right vagus was now cut and the pressure at once went up, reaching a height of 226 mm. Hg in one second. The pressure gradually went up to 232 mm. Hg during the 102 seconds following, and now the left vagus was cut, but no alteration occurred in the pressure curve except that the respiratory undulations became well marked. The pressure fell to its lowest point 180 seconds later.

Heart Beats.—When the right vagus was cut the heart was accelerated in rate by 24 beats per minute. No notable acceleration occurred after the left vagus was cut, but the heart beats gradually increased in frequency and just before the blood pressure finally fell they were 246 per minute.

Respirations.—The respirations were markedly affected on division of the vagi, becoming quite characteristic in type.

Experiment 8.—Cat, weight 2920 grammes. Poison 1/10 gramme (= 0·034 gramme per kilogramme).

Blood Pressure.—The blood pressure started to rise 20 seconds after the injection, and went up from 170 mm. Hg to 194 mm. Hg. Then followed the usual slight fall to 184 mm. Hg, and later a steady rise to a maximum of 224 mm. Hg 120 seconds after the injection. From this point the pressure steadily fell during the next 100 seconds to 30 mm. Hg. As the blood pressure was declining (200 seconds after injection) the left vagus was cut, but this procedure brought about no effect whatever upon the blood pressure. The right vagus was cut 10 seconds later with a similar result.

Respirations.—Although there was no effect upon the blood pressure, yet after both vagi had been divided typical vagus respiration ensued. Inspiratory gasps were taken for five minutes after the blood pressure had reached its lowest point.

Heart Beats.—Before injection the frequency of the heart beats was 168 per minute. Just before the final fall it was 270 per minute.

Experiment 9.—Cat, weight 2130 grammes. Poison 1/20 gramme (= 0·023 gramme per kilogramme).

Blood Pressure.—The injection of the poison occupied 15 seconds, and the blood pressure began to rise at the end of the eighth second. At the end of 20 seconds the pressure had risen from 136 to 140 mm. Hg to 166 to 168 mm. Hg. During the next 25 seconds several sudden falls of from 10 to 16 mm. Hg occurred. The pressure then became steady and commenced to rise slowly.

The right vagus was cut at this moment (54 seconds after the injection) and 19 seconds later the left vagus was cut. These sections produced no alteration in the steady rise of blood pressure, except such as were due to the altered respiratory rhythm.

The pressure reached its maximum point of 194 to 196 mm. Hg 131 seconds after the injection; it then commenced falling very rapidly, and reached 40 mm. Hg above zero in 40 seconds (three minutes after the injection).

Heart Beats.—During the first rise of blood pressure the pulse rate remained at 180 per second, the same as it was before the injection. During the next period of the curve, when the falls in pressure were taking place, the rate of the heart sank to 156 per minute. Later it became accelerated again (about 228 per minute). The two sections did not materially interfere with the frequency, which gradually increased up to 249 per minute when the blood pressure reached its height. It maintained this rate as the pressure commenced to fall. The beats had now become extremely small.

Respiration.—No change occurred in the respirations until the second vagus had been cut, and then the respirations became of the vagus type. The respirations became irregular and small as the heart stopped, and inspiratory gasps were taken at intervals for 100 seconds later.

Stimulation of Vagus.—Stimulation of the vagus 26 seconds after the second vagus had been divided produced no effect whatever upon the heart beats or blood pressure. No alteration was produced on again stimulating the nerve with the full strength of current when the blood pressure had attained its maximum point. A marked effect was, however, produced upon respiration, which stopped suddenly. A third stimulation with the full strength of current for a period of seven seconds was applied when the blood pressure had commenced falling, but no effect whatever was produced on the blood pressure or heart beats although the respiration stopped.

Experiment 10.—Cat, weight 2930 grammes, poison 1/20 gramme (= 0·017 gramme per kilogramme).

Blood Pressure.—The blood pressure began to rise at once, and from 132 to 146 mm. Hg it reached 164 to 174 mm. Hg in 32 seconds. After remaining stationary for 35 seconds it began to fall. When the pressure had fallen to 150 to 168 mm. Hg the left vagus was cut, with the result that the pressure immediately rose, and in 15 seconds reached 180 to 192 mm. Hg. A second fall now occurred to 166 to 174 mm. Hg, and finally the pressure rose again in a long curve to a maximum of 208 to 218 mm. Hg, and from this point it gradually fell till death. Section of the right vagus before the maximum point was reached produced no alteration in the pressure curve. The blood-pressure curve fell very gradually and had many irregular rises and falls on it towards the end. It reached 24 mm. Hg in 17½ minutes after the poison was injected, and at this point death occurred.

Heart Beats.—During the first rise the rate of heart beats was unaltered. Whilst the pressure was stationary and during the first fall the rate was slowed from 231 to 216 per minute. After section of the left vagus the rapidity slightly increased to 220 per minute, and during the second fall the rapidity was 214 per minute.

From this point the rapidity gradually increased to 255 per minute when the blood pressure was at its height. When the pressure was finally falling, 100 seconds later, the rapidity was still 255 per minute, and after this the pulsations were too small to count.

Respiration.—The respirations were very irregular during the whole time, and typical vagus respiration occurred after section of both vagi.

Inspiratory gasps continued for from two to three minutes after the heart had stopped.

Stimulation of the Vagi.—The vagi were stimulated 10 times, commencing just when the blood-pressure curve was starting on its rise to the maximum point and ending about two minutes before the heart stopped. On each occasion there was no effect whatever upon the blood-pressure curve or upon the heart beats.

Experiment 11.—Rabbit, weight 2210 grammes, poison 1/20 gramme (= 0·022 gramme per kilogramme). Both the vagi were divided before the poison was injected.

Blood Pressure.—The blood pressure rose from 124 to 130 mm. Hg to a maximum height of 168 to 170 mm. Hg during the 39 seconds after the poison was injected. Death occurred 84 seconds later.

Stimulation of Vagus.—The left vagus was stimulated (strength 10) just as the pressure curve reached its maximum height, but no effect was produced on the blood pressure. The vagus was again stimulated as the blood pressure was falling, but there was produced no effect upon the curve.

Experiment 12.—Rabbit, weight 3065 grammes, poison 1/5 gramme (≐ 0·065 gramme per kilogramme). Both vagi divided before injection.

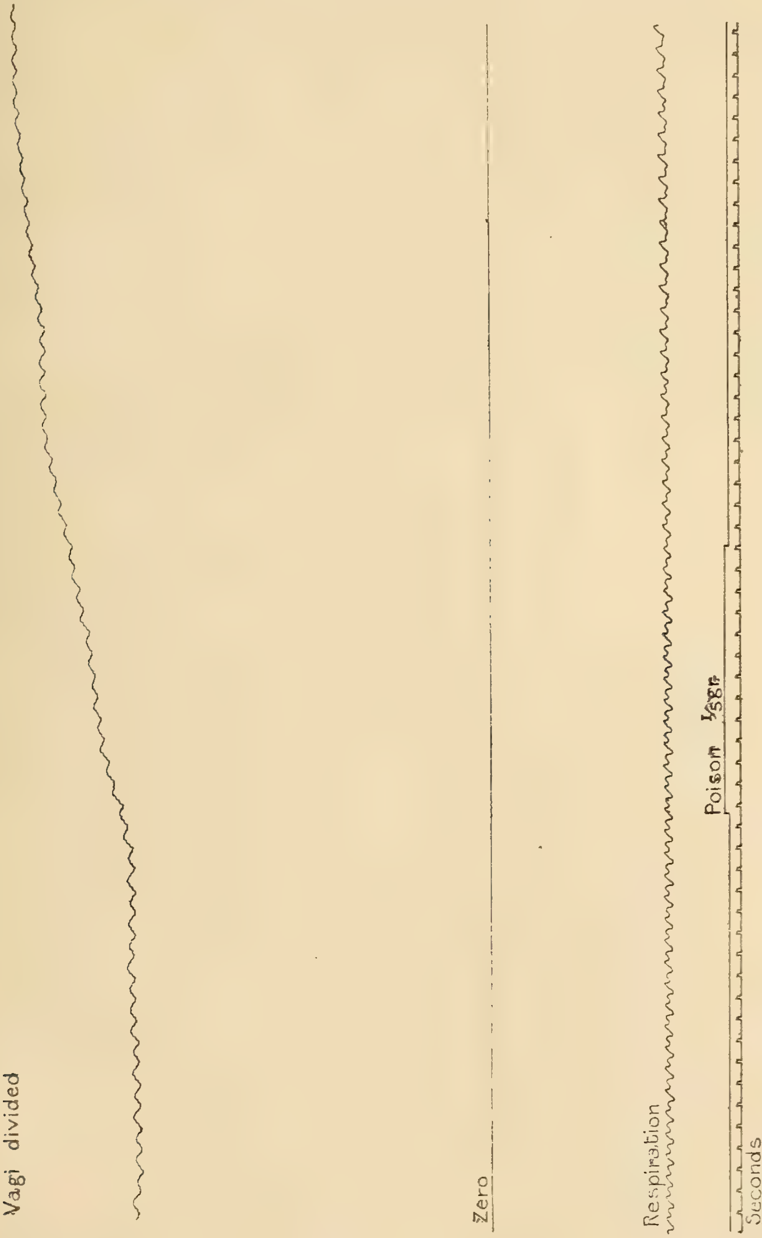
Blood Pressure.—The pressure immediately began to rise, and from 144 to 146 mm. Hg it rose to a maximum of 190 to 192 mm. Hg during the 27 seconds after the injection. This maximum height was maintained for 15 seconds, when the pressure commenced falling, and death occurred one and a half minutes after the poison was injected.

Stimulation of Vagus.—The left vagus was stimulated (strength 10) when the blood pressure had attained its maximum height. There was no direct effect upon the blood-pressure curve, but it was altered indirectly through the effect of the stimulation upon respiration. A second stimulation produced no result upon the blood pressure, although respiration was affected as before.

Section of Spinal Cord.

Experiment 13.—Cat, weight 2720 grammes. The spinal cord was divided at the level of the third cervical nerve. Artificial respiration was maintained at a rate of 39 per minute. Poison 1/20 gramme injected intravenously (= 0·018 gramme per kilogramme).

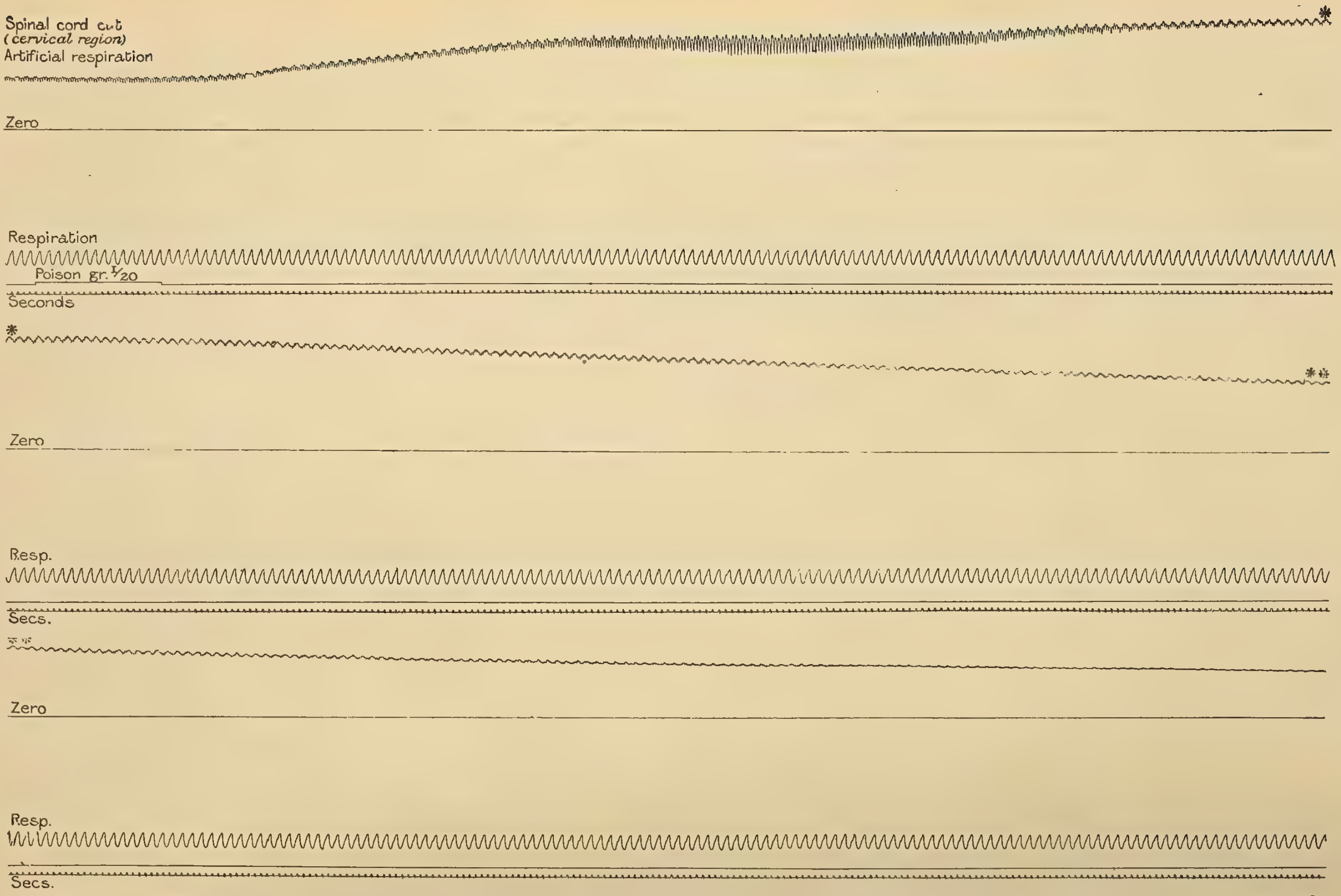
Blood Pressure.—The rise in pressure commenced 15 seconds after the injection was given, and from a height of 38 to 40 mm. Hg it attained a maximum height of 80 to 84 mm. Hg 200 seconds after the injection was given. From this point the pressure curve gradually fell till the heart stopped about seven minutes later. Before the pressure curve attained its



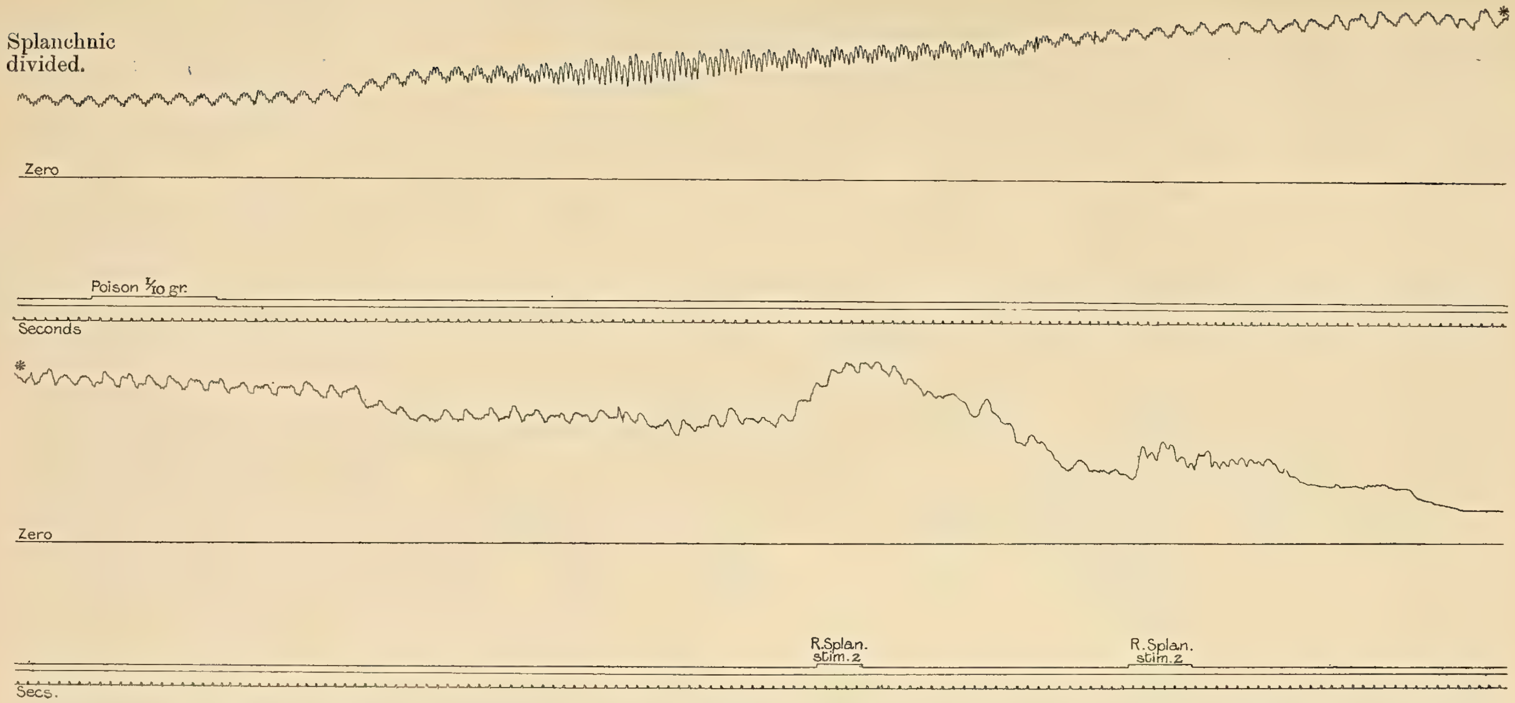
EXPERIMENT 12.—This tracing shows that the blood pressure still rises on injection of the poison after both vagi have been divided. It also shows that the slight fall, with slowing of the pulse, which occurs after the initial rise of blood pressure, does not occur when the vagi are divided.

maximum point it was interrupted by a very slight fall with slowing of the heart and increased size of the beats.

Heart Beats.—Before the injection was given the rate of the heart beats was 153 per minute. During the period of large heart beats the rate was 114 per minute, the size of the variations in pressure being 12 mm. Hg.



EXPERIMENT 13.—The spinal cord was cut previous to the injection of the poison in this experiment, and it is seen that, notwithstanding the section, the blood pressure rises and falls in the same manner as it does when the vasomotor system is intact.



Experiment 15.—The effect of injection of the poison after the splanchnic nerves have been divided is seen here. The blood pressure rises in a long curve as when these nerves are intact. The right splanchnic was stimulated twice as the pressure was falling, with a resulting rise in the pressure on each occasion, although only slight in the second case, as the heart was almost dead.

When the pressure curve was at its maximum height the rate was 204 per minute. The beats became much smaller and 228 in frequency as the pressure was falling. The last time the beats could be distinguished the rate was 280 per minute.

Experiment 14.—Cat, weight 2750 grammes. Spinal cord cut opposite third cervical nerve. Poison 1/20 gramme given twice (= 0·018 gramme per kilogramme).

The same sequence of events was observed as in Experiment 13. After a second injection of the same amount of poison during the period of large heart beats the pressure rose somewhat suddenly, the heart beats becoming smaller and showing a respiratory rhythm. Towards death the heart beats became irregular and rapid.

Section of Splanchnic Nerves.

Experiment 15.—Cat, weight 2470 grammes. Artificial respiration. The chest was opened on both sides, and both splanchnic nerves divided above the diaphragm. Poison 1/10 gramme injected (= 0·04 gramme per kilogramme).

Blood Pressure.—The blood pressure commenced rising 12 seconds after the injection was given. During the rise a period of slowing of the heart beats occurred as in the spinal cord experiments. This only lasted about 25 seconds, and slightly interrupted the rise of blood pressure. The maximum height of the blood pressure was 126 to 138 mm. Hg, and this occurred two minutes after injection, the blood pressure before injection being 56 to 64 mm. Hg. During the next 70 seconds the pressure gradually fell to 90 to 100 mm. Hg. The right splanchnic nerve was now stimulated (strength of current, 12), and the pressure in response to this immediately commenced rising and reached the height of 136 to 144 mm. Hg. In the next 25 seconds it gradually fell to 50 mm. Hg. The splanchnic nerve was again stimulated with the same strength of current, and the blood pressure rose to 80 mm. Hg. The heart now rapidly failed, and in 20 seconds had stopped, the pressure falling to 20 mm. Hg above zero.

Before injection the rate of the heart was 198 per minute. As the pressure was rising the rate fell to 117 per minute. When the maximum pressure was reached the frequency was 249 per minute. As the pressure fell the heart began beating irregularly and the rate was 255 per minute.

Previous Injection of Apocodein.

Experiment 16.—Rabbit, weight 2720 grammes. Apocodein 1/10 gramme injected (= 0·036 gramme per kilogramme). The blood pressure immediately fell from 84 to 50 mm. Hg. Stimulation of the sciatic nerve now produced no rise of blood pressure, showing that the peripheral terminations of the vasomotor nerves were paralysed. Poison 1/10 gramme (= 0·036 gramme per kilogramme) was now injected. The blood pressure went up to a maximum height of 120 mm. Hg and from this point the pressure fell as in all the other animals as the heart failed.

Experiment 17.—Rabbit, weight 2380 grammes. Both vagi divided. Blood pressure 124 mm. Hg. Apocodein 1/10 gramme injected (= 0·042 gramme per kilogramme). The blood pressure after a slight rise rapidly fell to 84 mm. Hg. Stimulation of the sciatic nerve produced a slight rise of 4 mm. Hg and therefore a second injection of the same dose of apocodein was administered. After this, stimulation of the sciatic nerve produced no rise in the blood pressure, which remained at 84 mm. Hg.

Poison 1/10 gramme (= 0·042 gramme per kilogramme) was now injected. At the end of the injection the blood pressure commenced to rise steadily to a maximum of 160 mm. Hg. From this point the pressure fell in the usual way, the action of the heart being irregular. The sciatic nerve was stimulated four times after the poison was injected and the slight alterations occurring in the blood-pressure curve are secondary to the alterations in the respirations.

Stimulation of the Depressor Nerve.

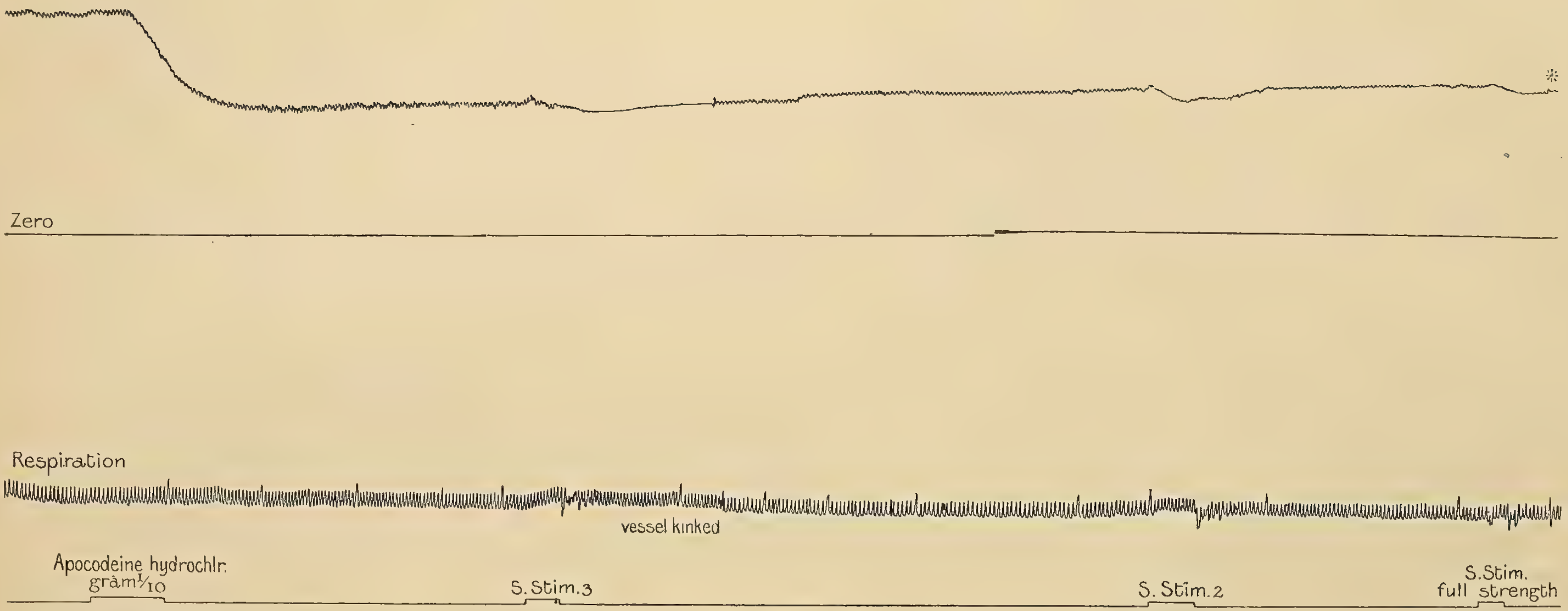
Experiment 18.—Rabbit, weight 2650 grammes. Left depressor nerve cut. Poison 1/30 gramme was injected intravenously (= 0·012 gramme per kilogramme).

Blood pressure.—After the pressure had risen in the usual way as the result of the injection, the central end of the depressor nerve was stimulated three times, the last stimulation being applied five minutes after the poison was injected. On each occasion the blood pressure fell to the same extent as it did on stimulation of the depressor before the poison was injected.

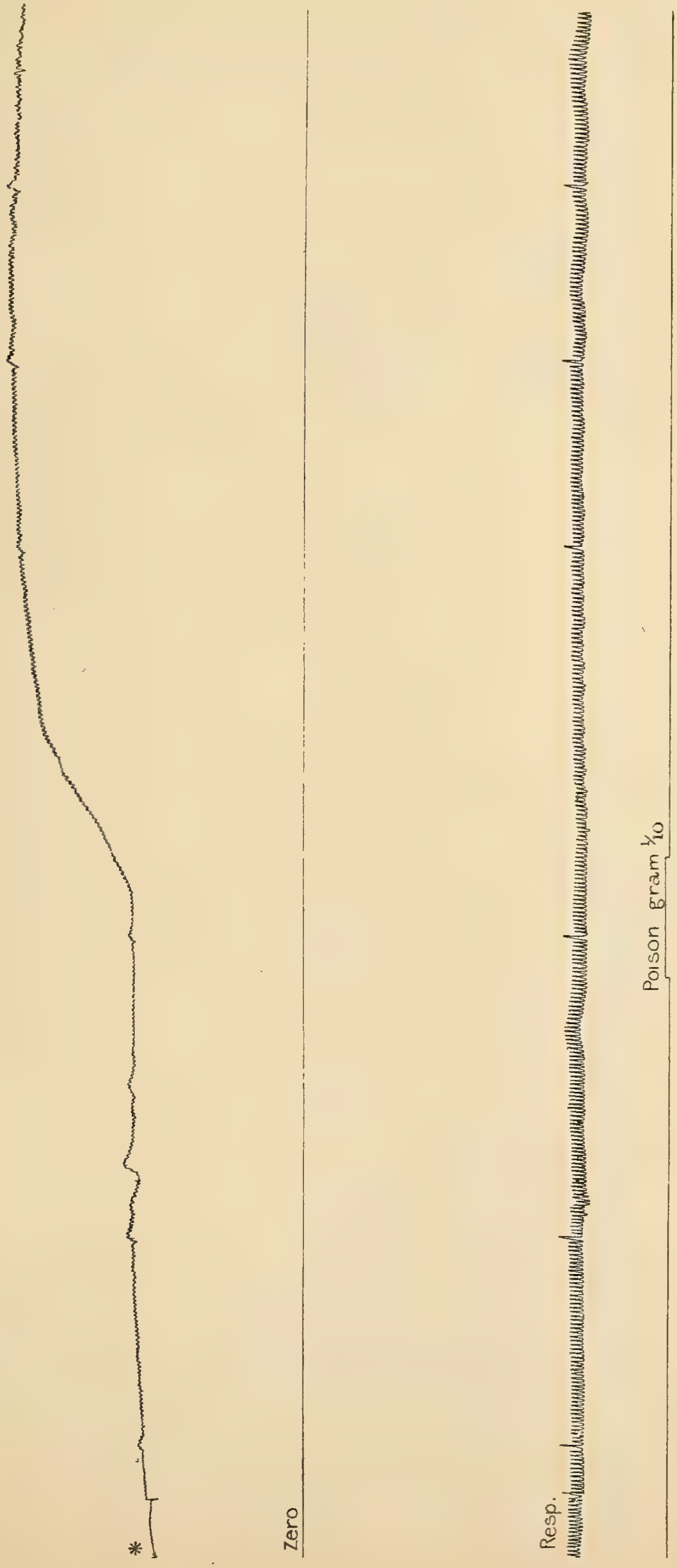
A second injection of poison (1/30 gramme) was administered six minutes after the first. After the blood pressure had risen as the result of the injection the depressor was stimulated five times.

The last stimulation was applied six minutes after the second injection. On each occasion except the first, when the stimulation was applied just as the blood pressure was commencing to rise as a result of the injection of the poison, the fall of pressure was practically to the same degree.

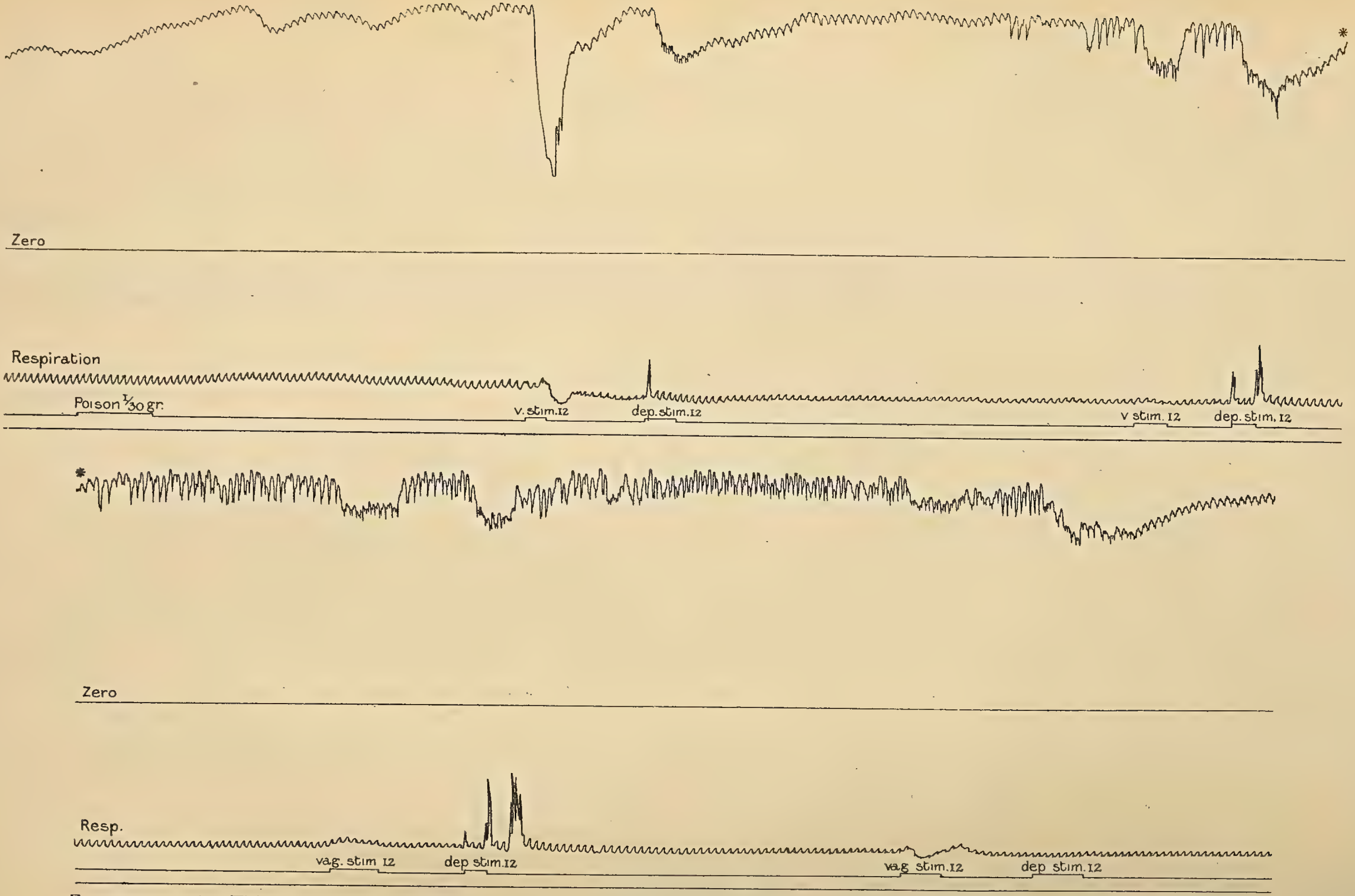
When the last two stimuli were applied the blood pressure had commenced to fall and the heart to beat irregularly. A third injection of poison (1/30 gramme) was given before the blood pressure had reached



Experiment 16.—In this case apocodein was injected and, as is seen in the tracing, produced a fall in the blood pressure. Stimulation of the central end of the divided sciatic nerve now produced no reflex effect upon the blood pressure. On injection of the poison, however, the blood pressure went up in the usual manner.



EXPERIMENT 16—*continued.*



Experiment 19.—This tracing shows the effect of four excitations of the vagus, the depressor nerve being stimulated immediately after the vagus on each occasion. The same strength of current was used for each stimulation. The effect of the vagus stimulation becomes rapidly less, but that of the depressor remains practically the same. If a larger dose of poison is used the vagus ceases to respond much earlier.

its lowest point and no rise of pressure resulted. Stimulation of the depressor three times still caused a fall in the pressure even at this late stage. The falls, however, were less marked than the former ones.

Experiment 19.—Rabbit, weight 2400 grammes. Left vagus cut. Poison 1/30 gramme injected intravenously (= 0·013 gramme per kilogramme).

Blood pressure.—After the pressure had risen the vagus was stimulated at quite an early stage (strength 12). The pressure at once fell from 136 mm. Hg to 40 mm. Hg. The depressor was stimulated immediately afterwards (strength 12) and the pressure fell from 132 to 136 mm. Hg to 104 to 108 mm. Hg. The vagus, and immediately afterwards the depressor were stimulated three times subsequently with the same strength of current. The vagus effect became each time less marked, on the third occasion the pressure only falling 10 mm. Hg. The depressor effect, however, remained the same till the end.

A second injection of poison was administered and stimulation of the vagus on three occasions produced no effect whatever, although the strength of the current was increased. Stimulation of the depressor, however produced a fall in the blood pressure of 20 mm. Hg just before the animal died.

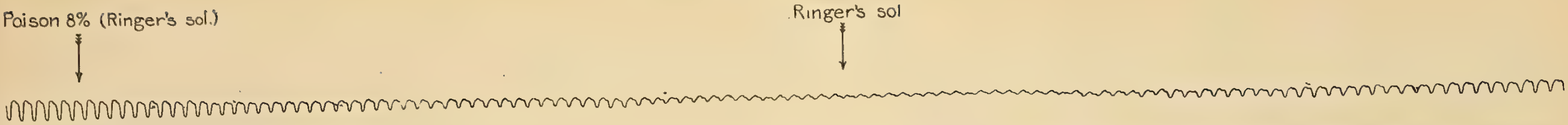
III. *Frog Heart Experiments.*

Experiment 1. *Pithed frog.*—The poison was dissolved in Ringer's solution (1/15 gramme to 1 c.c.) and the heart kept moist by occasionally applying the solution with a brush. The rate of the heart beats before the application was 42 per minute. During the application the rate finally sank to 30 per minute, the force of the ventricular systole being much weaker than that of the auricle. The ventricle occasionally took on an independent rhythm and its beats gradually became weaker and weaker till it finally stopped in diastole, the auricle continuing to beat after the ventricle had stopped. The auricle finally stopped and washing with Ringer's solution failed to restore the heart.

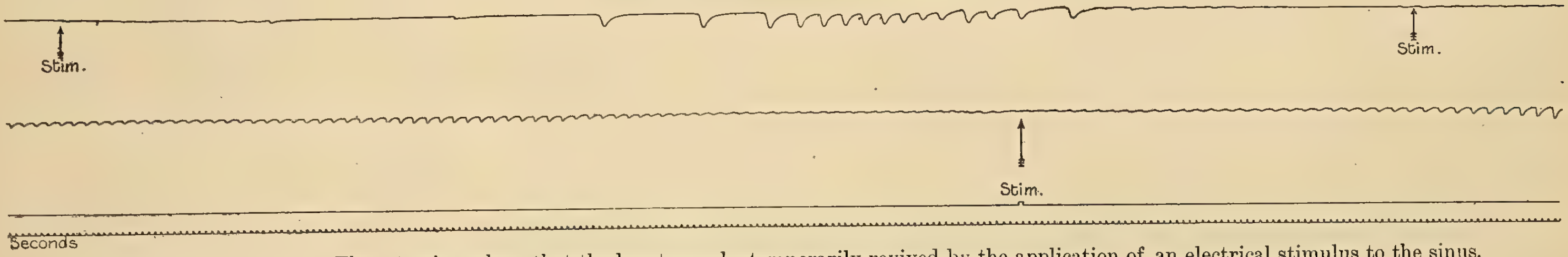
Experiment 2. *Pithed frog.*—Poison 8 per cent in Ringer's solution. When the heart had almost stopped the poison was washed off with Ringer's solution and the heart kept constantly moist with it. The heart finally recommenced to beat in the normal manner.

Experiment 3. *Pithed Frog.*—Poison 1/5 gramme to 1 c.c. salt solution. The contractions of the ventricle gradually became smaller and smaller, the rate declining from 42 to 36 per minute, and it finally stopped in diastole. Whilst the auricle was still contracting the sinus was stimulated with an induced current. Complete standstill resulted for about 25 seconds and then

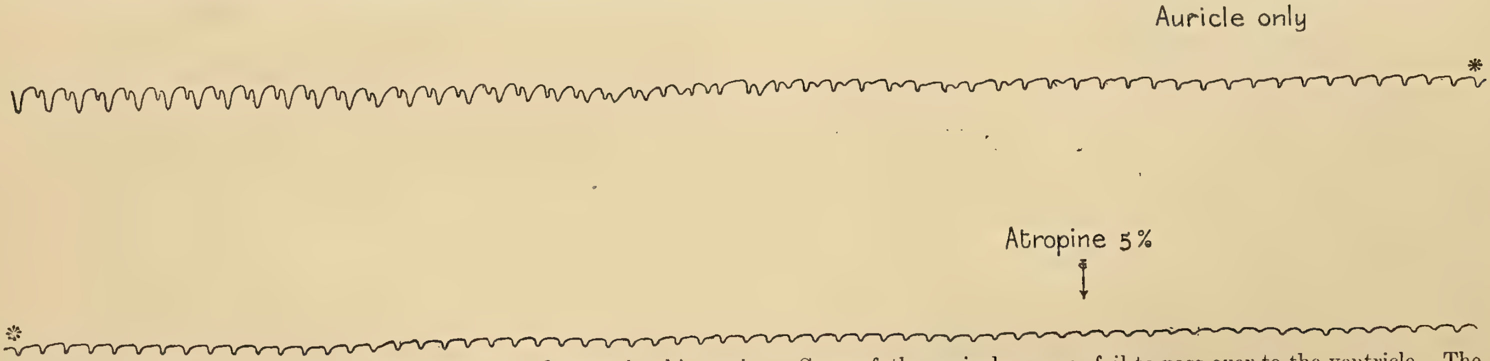
FROG HEART EXPERIMENTS.



EXPERIMENT 2.—This tracing shows the gradual stoppage of the frog's heart under the influence of the poison, and its revival by washing with Ringer's solution when the ventricle has almost stopped.

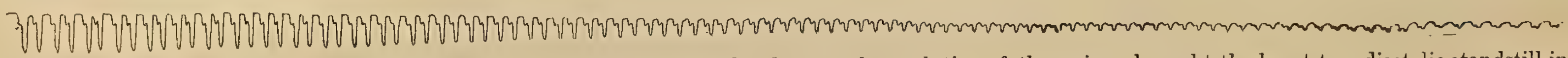


EXPERIMENTS 3 AND 4.—These tracings show that the heart may be temporarily revived by the application of an electrical stimulus to the sinus.



EXPERIMENT 5.—The auriculo-ventricular block can be seen in this tracing. Some of the auricular waves fail to pass over to the ventricle. The ventricle finally stops and the auricle goes on beating. Application of atropine fails to produce any effect and the auricle stops in diastole.

Atropine 5 %.
Poison 8 % 70 seconds later.



EXPERIMENT 6.—In this case atropine was first applied to the heart, and subsequently a solution of the poison brought the heart to a diastolic standstill in spite of the action of the atropine.

the heart commenced beating again, the beats gradually increasing until they reached their former size. The rate was 27 per minute.

On the application of more of the poisoned solution, the heart again stopped. A second stimulation after a longer latent period than before caused the heart to recommence beating. This time the beats did not attain their former size. Rate, 21 per minute, the beats being somewhat irregular. The heart soon stopped on its own accord, and a third stimulation restarted the contractions, which gradually died away, and now a further stimulation failed to restore the contractions.

Experiment 4. *Pithed Frog.*—Poison, 1/10 gramme to 1 c.c. Ringer's solution. The heart was stopped except for a tiny and feeble contraction of the auricle by application of the poison. A stimulus applied to the sinus as in Experiment 3 restored the heart beats. When the beats had attained their normal size, the application of more poison stopped the heart and stimulation now failed to arouse it.

Experiment 5. *Pithed Frog.*—Poison, 8 per cent. in Ringer's solution. The first effect of the poison was a gradual diminution in the size of the ventricular beats until they became the same size as the auricular beats and finally smaller. At this stage slow peristaltic action of the ventricle occurred, passing from base to apex. The ventricle also dropped beats and thus a marked irregularity of action was produced owing to a block at the auriculo-ventricular junction. On discontinuing the application of the poison the ventricle recovered itself.

More poison was applied and again the ventricle showed the same action as before and finally recovered a second time on the application being discontinued. A third application of the poison brought the ventricle to a standstill, the auricle continuing to beat. A 5-per-cent. solution of atropine was now applied.

There was no result for a little while and then the rate of the auricular contractions became much slower, and finally the rate sank from 39 to 12 per minute. The heart was not restored.

Experiment 6. *Pithed Frog.*—Atropine, 5 per cent. in Ringer's solution applied. The heart was now beating at 33 per minute. After 70 seconds an 8-per-cent. solution of the poison (Ringer's solution) was applied. The heart continued to beat at the same rate for about 40 seconds, and then the rate increased to 42 per minute, the beats becoming smaller; when the ventricular beats had become the same size as those of the auricle the rate was 33 per minute. The ventricle stopped in diastole, the auricle continuing to beat for a short time, when the heart finally stopped.

Experiments 7, 8, 9, 10, 11.—Five more experiments with the frog's heart were done, but these will not be given in detail as the results were exactly the same as has already been described.

Experiment 12. *Nerve Muscle Preparation of Frog. Pithed Frog.*—The preparation was made in the usual way. An induced current was used to stimulate the nerve. The solution of poison was applied to the muscle with a brush. Each time the poison was applied the muscle shortened slightly. The muscle was kept moist with the solution of poison until it failed to contract, when the nerve was stimulated. The electrodes were now applied to the muscle itself, but it failed to respond with the strongest current.

On the Origin of the Sertoli or Foot-cells of the Testis.

By C. E. Walker, Assistant-Director of Cancer Research Laboratories, University of Liverpool, and Alice L. Embleton, B.Sc.

(Communicated by Dr. C. S. Sherrington, F.R.S. Received January 16,—Read February 1, 1906.)

[Plates 5 and 6.]

The function of the Sertoli or foot-cells of the mammalian testis has frequently been described, and will not be dealt with in the present brief communication. Neither do we intend to deal with the later stages of the life-history of these cells, except in so far as to draw a parallel between them and the cells performing a similar function in the amphibia.

In the embryo testis of a mammal before the tubules are formed it is seen that a number of so-called male ova lie, singly or in groups of about four, among masses of cells, which we will for the moment designate as being of a more or less undifferentiated character. These undifferentiated cells are much smaller and of quite a different character to the male ova (see fig. 1).

At a little later stage we find that the wall of the tubule begins to appear. This wall is apparently actually in process of formation in parts of fig. 1, while in fig. 2 a tubule with a complete wall has been formed. We are convinced from a careful study of the stages of development that cells which form the wall of the tubule and those that are enclosed with the male ova inside the wall thus formed are identical or derived from the same immediate ancestors. As development goes on these cells become more and more differentiated until we reach the state of the tubule in the adult testis (fig. 3).

The processes that are gone through in the testis of *Triton* seem to offer a further confirmation of the conclusions suggested above. Here the normal course of events in the male ova seems to be: (1) amitosis; (2) somatic mitoses; (3) the maiotic phase; and (4) the conversion of the cells resulting from the second maiotic (homotype) division into spermatozoa. These periods are sharply defined and are easy to follow. While amitosis is taking place, the individual male ova seem to be scattered amongst a number of "undifferentiated" cells, just as happens in the embryo testis of mammals. At this time the future pockets are but ill-defined or not defined at all. Apparently each individual male ovum eventually gives rise to a pocket (fig. 4). A little later on we see that the cells surrounding the male ova wander in between them as they multiply amitotically, until a stage is arrived at when it is impossible to say whether a particular one of them is going to become a cell forming the wall of a pocket or one of the cells which is enclosed within that wall together with the male ova (figs. 5, 6). To the latter the spermatozoa eventually become attached.

It will thus be seen that what happens in the development of the embryo testis of the mammal is parallel with what happens every year in the testis of *Triton*. Certain undifferentiated cells which surround the male ova are more or less differentiated along different lines, some apparently becoming cells to which the spermatozoa are attached during a certain period of time, others forming the walls of the tubules or pockets as the case may be.

It will be found that there are, among the undifferentiated cells in the early embryo testis of mammals, forms of division where the chromosomes are apparently reduced in number and different in shape to what is seen in ordinary somatic division (figs. 7, 8, and 9). These closely resemble what has been described as a second maiotic (homotype) division figure. It has been shown by one of us* that this form of division is very

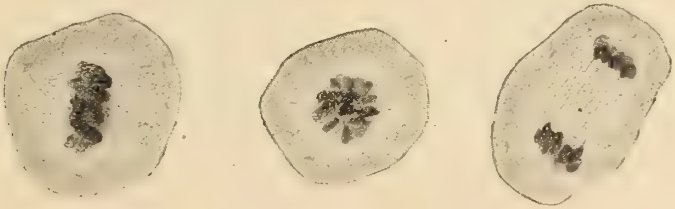


Fig. 7. Fig. 8. Fig. 9.

Mitotic figures in undifferentiated cells surrounding the male ova.

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* C. E. Walker, "Observations on the Life-History of Leucocytes" (next paper, p. 53).

common in leucocytes, in bone marrow, and lymphatic glands. While we have occasionally seen somatic division figures among these undifferentiated cells of the embryo testis, this form of reduced division is by far more common. In the male ova themselves at this stage the only mitotic figures we have seen have been typically somatic, and it is hardly possible to confuse the one kind of cell with the other.

The fact that we have what are apparently second maiotic divisions in cells which are obviously not destined to become sexual cells is in itself very suggestive, but when we come to compare the cells in which this happens with cells that are undoubtedly leucocytic in character, the suggestion is very much strengthened (figs. 10 and 11). We use the term leucocyte in the widest sense, and under it include all the wandering cells of the body.

Our conclusion is, therefore, that the foot-cells of the testis and the cells forming the walls of the tubules or pockets have immediately common ancestors, and that if these cells are not identical with certain stages in the series of leucocytic generations, they are derived from cells that were identical not more than two or three generations before.

DESCRIPTION OF PLATES.

Plate 5.

Fig. 1.—Early stage in the development of the testis. Signs of the wall of the tubule are visible in places. A division figure among the undifferentiated cells is shown. (Embryo guinea-pig.)

Fig. 2.—Later stage of the same. The wall of the tubule is formed.

Fig. 3.—Part of a tubule of adult testis, showing foot-cells. (Guinea-pig.)

Plate 6.

Fig. 4.—Early stage in the testis of *Triton.* The walls of the pockets are not everywhere defined.

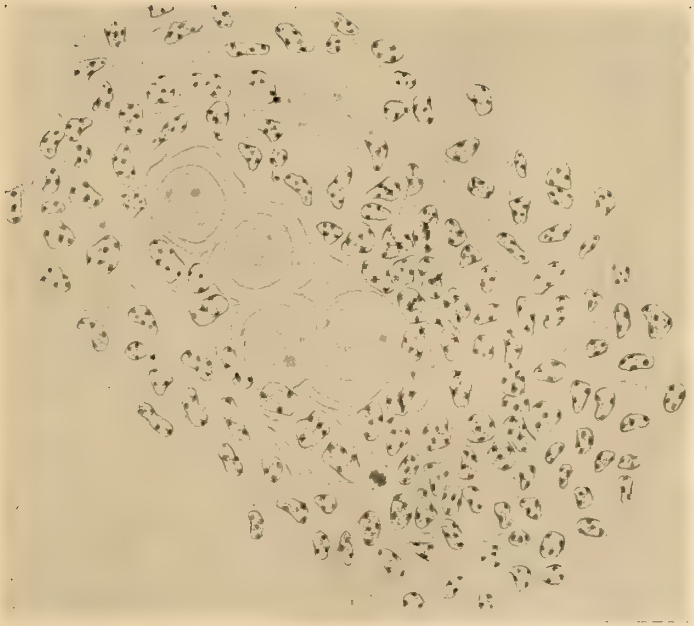
Fig. 5.— The same, showing the undifferentiated cells wandering in among the male ova.

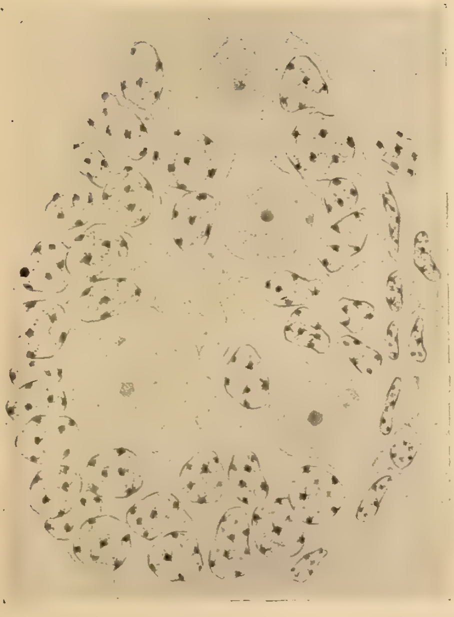
Fig. 6.—The same, showing pockets in process of differentiation and one completely formed.

Fig. 10.—Leucocytes in the lymphatic gland of guinea-pig.

Fig. 11.—Leucocytes in the spleen of *Triton.*

FIG. 1.





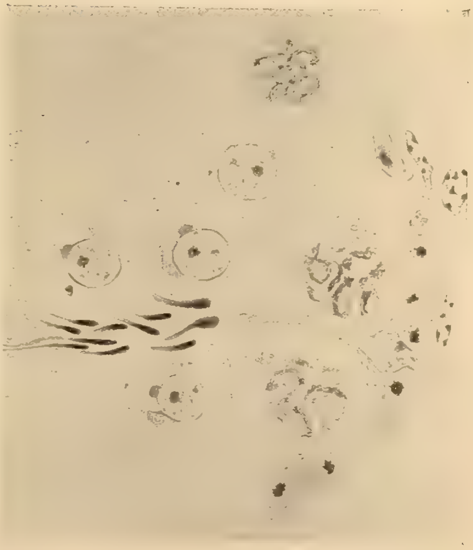


FIG. 2.

FIG. 3.

Walker and Embleton. *Roy. Soc. Proc., B. vol.* 78, *Plate* 6.

Fig. 4.

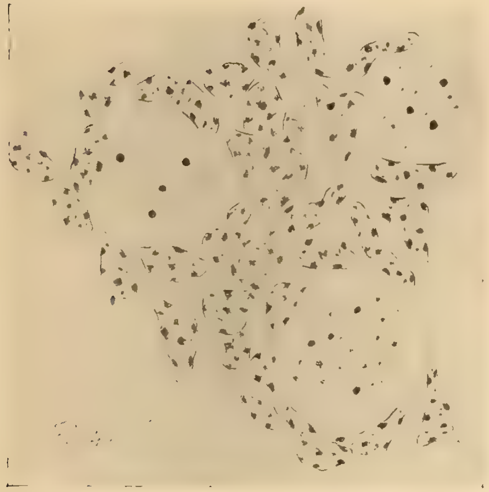
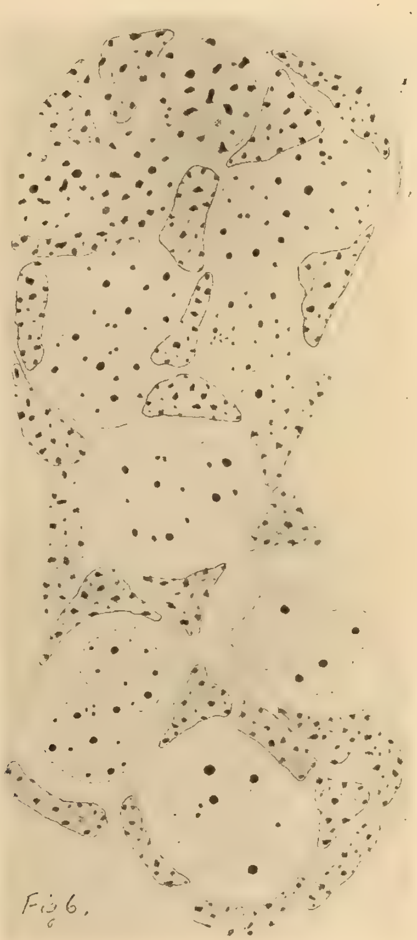


Fig. 6.



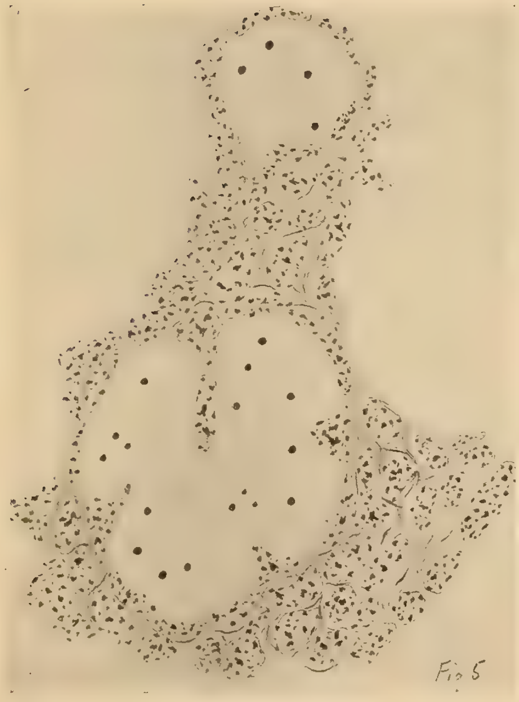


Fig. 5.

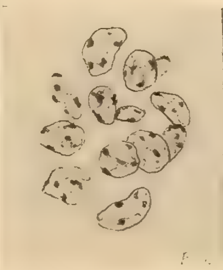


Fig. 10.

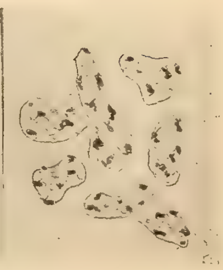


Fig. 11.

Observations on the Life-History of Leucocytes.

By C. E. Walker (Assistant-Director of Cancer Research Laboratories,
University of Liverpool).

(Communicated by C. S. Sherrington, F.R.S. Received January 9,—Read
January 18, 1906.)

[Plates 7—10.]

In presenting this preliminary communication it is necessary to explain that the term leucocyte is used in the widest sense, and is intended to include all the wandering nucleated cells and their immediate ancestors. After many endeavours to make the observations here recorded compatible with the current classification of these cells, it has been found imperative to use a term that will include them all, and, at any rate in most cases, to put aside for the moment the question as to which particular kinds of leucocyte are being dealt with.

The tissues used have been chiefly bone-marrow, lymphatic glands and spleen, but the leucocytes in various other tissues and the free leucocytes in the blood have also been examined. Most of the work has been done with material derived from the guinea-pig and the rat, but tissues from man, rabbit, mouse, crocodile, frog, *Triton* and *Axolotl* have also been used. In every case the tissues have been normal and, with one exception, derived from adult or nearly adult animals, the one exception being the testis of the early embryo of the guinea-pig.

In examining a section of bone-marrow one of the most striking, constant and frequent objects met with is the giant-cell or myeloplax. In the myeloplaxes there is, as a rule, an appearance of amitosis in the nucleus or nuclei (see fig. 1).

Occasionally, however, a myeloplax is found in which mitosis is taking place, and in such cases the mitotic figure is pluripolar but otherwise of the somatic type (see fig. 2). A further search shows that myeloplaxes with the chromatin of the nuclei in the ordinary somatic spireme form are not uncommon (see fig. 3). On the other hand, it is seen that the majority of the myeloplaxes divide amitotically. A portion or portions of the cell containing one or more nuclei are then separated off (see fig. 4).

Following the cells thus produced, one is forced to conclude that many of the smaller cells in the bone-marrow are derived from them. Among these are the cells commonly known as "polymorphic nuclear leucocytes" and many of the cells containing a single and more or less rounded nucleus (see fig. 5).

The polymorphic nuclear cells apparently continue to divide amitotically for a number of generations. It is impossible in the present brief communication to illustrate all these stages, but fig. 5 shows two of the most striking.

At present it is impossible to discriminate between the cells arising amitotically and those arising mitotically from the myeloplaxes.

Among the mononuclear cells of the bone-marrow and the germinal areas of lymphatic glands, mitotic division figures are very frequent. They are also present, but less numerous, among cells found in the blood and the spleen.

Many of these mitotic figures are of the ordinary somatic type, and nuclei showing the usual somatic spireme are not uncommon (see figs. 6, 7, and 8).

More rarely a division figure such as those shown in figs. 12, 13, 14, and 15 is met with. In many of these it is extremely difficult to make out even a single individual chromosome. In others, however, several can be made out quite distinctly. This is the case in figs. 12, 13, and 14. In fig. 15 the chromatin masses are much less clear, but even this is more distinct than is the case in a considerable proportion of such cells. Though it is not easy, on account of the small size of the cells and the difficulty of obtaining good fixation, to count the chromosomes in even a few among a great many cells, in those where anything approaching an estimation of the number is possible it is evident that the number is much smaller than that found in the somatic division figures occurring in the same animal. That reduction in the number of chromosomes takes place in leucocytes has already been pointed out in a previous communication.*

It would seem, on comparing these figures with fig. 16, which is the first maiotic (heterotype) division from the testis of a guinea-pig, that these two forms of division are similar to each other, but vastly different from the normal somatic division figures found in the same animal (see fig. 7).

The probability that these division figures are similar to that occurring in the first maiotic division in reproductive cells does not, however, depend only upon the forms of the chromosomes,† their number, or their arrangement upon the spindle. Prophases such as those shown in figs. 9 and 10 are not infrequent. In these the so-called spireme is seen to be distinctly split and to differ in other ways from the ordinary somatic spireme (see fig. 6). In fig. 11 a corresponding stage in the prophase of the first maiotic division in the testis of a guinea-pig is given as a comparison.* Beyond this again one occasionally finds a cell in the diaster stage in which some at any rate of the chromosomes are longitudinally split (see fig. 17). This is typical of the first maiotic division.† In so far as the leucocytes are concerned, I have hitherto found this type of division only in the bone-marrow.

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* Farmer, Moore and Walker, "On the Cytology of Malignant Growths," 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 336.

† It may here be pointed out that several of the permanent forms of first maiotic (heterotype) chromosomes described by Moore and Arnold have been observed in the division figures dealt with here. (Moore and Arnold, "On the Existence of Permanent Forms among the Chromosomes of the First Maiotic Division in Certain Animals," 'Roy. Soc. Proc.,' 1906.)

The typically somatic division figures and those that I have interpreted as heterotypes, however, even when taken together, are less numerous than another form of division of which figs. 18 to 23 are illustrations. This form of division is extremely common in bone-marrow and in lymphatic glands. Hitherto I have not found it in any other adult tissue or in any other tissue that I have examined, with one exception referred to later.

It is obvious, as will be seen from the figures, which are taken from cells occurring in the bone-marrow and lymphatic glands of the guinea-pig and rat, that the number of chromosomes is not more than half the normal somatic number, which is 32 in both cases. The shape of the chromosomes seems to vary slightly in different cells. Some are short, thick, and slightly curved rods (see figs. 18 and 22). Others are more or less oval, often very irregular. There seems to be every reason for regarding these divisions as similar to the second maiotic (homotype) division. Examples of this division from the testis of the guinea-pig are given in figs. 24, 25 and 26. The great frequency with which this division occurs suggests strongly that there are several generations of cells showing the reduced number of chromosomes. The fact that in many cells the chromosomes are rod-shaped while in others they are roughly oval, led me at first to believe that there might be a well-defined difference between the first and the succeeding generations after the first maiotic division. An examination of the testes of several animals, however, convinced me that the same difference might be demonstrated among the homotypes there (see figs. 24 and 25). How far this is to be regarded as a real difference or merely the result of a presentation of the cell to the eye or to faulty fixation in some cells is here beside the point, as we know that in these particular animals there is no further division after the second maiotic (homotype).‡

In comparing the foregoing observations with what occurs in the normal production of sexual elements in animals and plants, some remarkable similarities become evident. Amitosis occurs as one of the earliest known phenomena in the production of spermatozoa in some animals if not in all.* The first maiotic is, in spermatogenesis, immediately preceded by the ordinary somatic form of division. Then follows the second maiotic division, retaining half the somatic number of chromosomes as in the first maiotic. No further division has been recorded in the case of animals. Except as regards the latter point, what happens among the cells of the bone-marrow seems to be in many respects parallel to what happens in those of the testis, for the myeloplaxes very possibly, probably as I believe, correspond to the more or less syncytial condition of the cells sometimes observed in vertebrate testes, both arising amitotically.†

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* Farmer and Moore, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants," 'Quart. Journ. of Mic. Science,' vol. 48, Part IV, February, 1905; Moore and Walker, "The Maiotic Process in Mammalia," 'Thompson-Yates Reports,' University of Liverpool, 1906.

† Farmer and Moore, Moore and Walker, *loc. cit.*

‡ Farmer and Moore, Moore and Walker, *loc. cit.*

In plants, however, we find that though the series of complicated cell phenomena that occur in the production of sexual elements are practically identical to what occurs in animals, other cells than those destined to become mature sexual cells are involved in the maiotic phase. Without going into details that would here be out of place, it may be pointed out that in many cases in plants but comparatively few of the cells that pass through the first maiotic division, and thereafter show but half the somatic number of chromosomes, ever become converted into sexual elements, and also that the number of post-maiotic generations (those following the second maiotic or homotype division) is often very great, even if they can be considered in many cases as having any definite limit at all.

If the observations recorded above be correct, it would seem that the life-history of the leucocytes, in so far as I have been able to follow it, shows some remarkable points of resemblance to the life-history of those reduced cells in plants to which I have just referred. This comparison is carried even further by what has been observed with regard to the origin and history of the foot-cells of the testis.‡ All the observations recorded above were made upon adult tissues, but in the course of seeking for the origin of the foot-cells I examined the testes of very early embryos of the guinea-pig. Here, long before the formation of the tubules, the cells that are destined to become foot-cells are practically indistinguishable from certain of the stages observed in the leucocytes found in the bone-marrow and lymphatic glands of the adult animal. In these cells, besides the ordinary somatic division figures, others are found exactly similar to those that I have described as second maiotic and post-maiotic in leucocytes (see figs. 27 and 28). In *Triton* testes it is possible to trace stages between the cells that have always been regarded as, and probably are, connective tissue cells and the cells that apparently perform the same function as the foot-cells of mammalian testes. In *Triton* also, during the earlier stages of the maiotic phase, the cells that are destined to become foot-cells are similar to certain leucocytes in the same animal. Accepting these observations as correct, and regarding the pockets of the amphibian testis as being directly comparable with the tubules of the mammalian testis, I am forced to the conclusion that either the leucocytes themselves or their immediate ancestors may give rise to connective tissue, the former probably being what really happens.

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* Meves, 'Anat. Anz.,' 1891, No. 22; 1894, 'Arch. m. Anat.'; Moore and Walker, *loc. cit.* Observations contained in a paper, not yet published, by Miss Embleton. They were carried out in the laboratories of the Cancer Research, University of Liverpool.

† Moore and Walker, *loc. cit.*

‡ Walker and Embleton, "On the Origin and Life-history of the Sertoli or Foot-cells of the Testis" (p. 50, *supra*).

While, as has already been said, there are remarkable points of similarity between the life-histories of the leucocytes and those cells in plants which, though reduced, never become converted into sexual elements, it is also evident that there are some important points of difference. In plants, both the cells just referred to and those which are converted into definite sex cells have commenced the maiotic phase at the same time, and their immediate ancestry is common to both. In the case of leucocytes and certain connective tissues it is not at present demonstrable that anything of the kind happens. It may be that our present conception of what constitutes the whole of the maiotic phase, in animals at any rate, is too limited, and that the development of the mesoblast is in some way involved in its earliest stages. In this connection the fact that in some plants reduced cells may be differentiated into tissues that are somatic in characters and function is extremely suggestive, as are also the observations of Loeb upon the segmentations and the production of embryos in unfertilised eggs.

It does not seem out of place to mention here the bearing that these observations have upon what happens in cancer. As has been shown elsewhere,* one of the earliest phenomena observed in the development of cancer is the fusion of a leucocyte with a tissue cell and the subsequent division of the cell resulting from the fusion into two daughter cells, each possessing chromatic elements derived partly from the leucocyte and partly from the tissue cell. That in the subsequent generations of the cells produced from this fusion the characters of both ancestors should appear is exactly what would be expected. Among the cells of malignant growths all the forms of division here recorded as occurring among leucocytes and their immediate ancestors are to be found. As has also been stated before, some of the

* Farmer, Moore and Walker, "On the Behaviour of Leucocytes in Malignant Growths," 'Trans. of the Path. Soc. London,' vol. 56, Part III, 1905.

cells in malignant growths apparently go on dividing mitotically for a number of generations with the reduced number of chromosomes.*

Note.—January 8, 1906.—The following fixatives have been used and in every case the results described have been found with all the fixatives. Flemming's Fluid (strong formula), Hermann's Fluid, Acetic Acid and Absolute Alcohol, Corrosive Sublimate and Acetic Acid, and strong Formic Acid. As it is possible that these observations may interest some who are not conversant with cytological methods, it is perhaps permissible to point out that the greatest care must be taken with the processes of fixation, dehydration, imbedding, staining, etc. Extremely small pieces of tissue should be placed in the fixative within about a minute of the death of the animal or removal from the living body. The dehydration should be carried out in short stages, an increase of 10 per cent. of alcohol at a time being perhaps best. This does not of course apply to the tissues fixed in Acetic and Alcohol or strong Formic Acid (40 per cent.), from which the tissues are transferred immediately to absolute alcohol. At the same time it is necessary that the tissues should not be left in under 80 per cent. of alcohol for more than two or three hours after fixation. In imbedding, no higher temperature than 45° Centigrade should be used. These remarks apply particularly to mammalian tissues, but also to all animal tissues. Throughout the process of staining and mounting the greatest care must be taken that the sections do not become even partially dried upon the slides.

It is almost necessary to use a 10-inch tube microscope with a monochromatic light. I have used apochromatic objectives and eye-pieces specially constructed for the long tube by Zeiss. With a monochromatic light it is possible to obtain excellent definition with a 27 or even 40 compensated ocular and a 2 or 3 mm. apochromatic objective. Anything approaching this is impossible with the ordinary short tube.

In view of the enormous advantage gained by using a monochromatic light, the stains must be chosen with regard to the colour of the light used. The part of the spectrum between the blue and the green gives the shortest wave-lengths that can be conveniently used. As this gives a better definition than the parts of the spectrum with longer wave-lengths, red, yellow and orange stains give the best results.

DESCRIPTION OF PLATES.

Plate 7.

Fig. 1.—Myeloplax from bone-marrow of guinea-pig, showing nuclei apparently dividing amitotically.

Fig. 2.—Ditto, showing pluripolar spindle figure.

Fig. 3.—Ditto, showing two nuclei with somatic spiremes.

Plate 8.

Fig. 4.—Ditto, showing nuclei that have divided and the cytoplasm dividing (amitosis).

Fig. 5.—Adjacent cells in the bone-marrow of guinea-pig, showing stages of differentiation.

Fig. 6.—Mononuclear cell from bone-marrow of guinea-pig. The somatic spireme is formed.

Figs. 7 and 8.—Later stages in the somatic type of division in similar cells.

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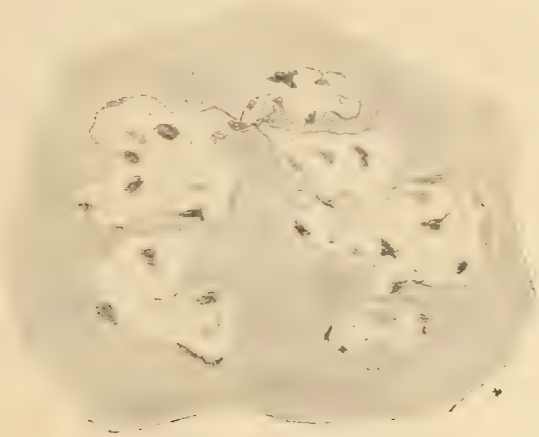
* Farmer, Moore and Walker, "On the Resemblances exhibited between the Cells of Malignant Growths in Man and those of Normal Reproductive Tissue," 'Roy. Soc. Proc.,' December, 1903.

Fig. 1.



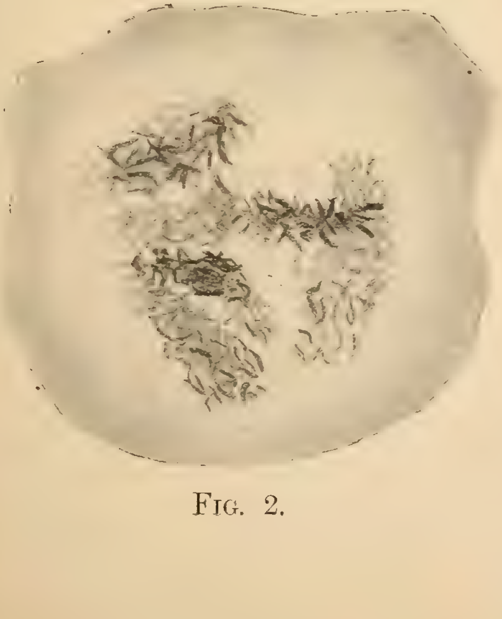


Fig. 2.

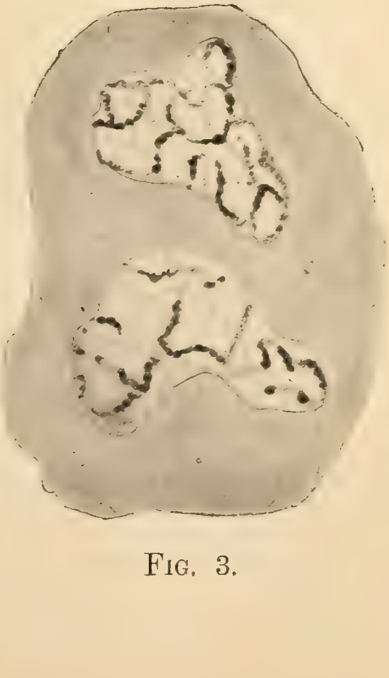
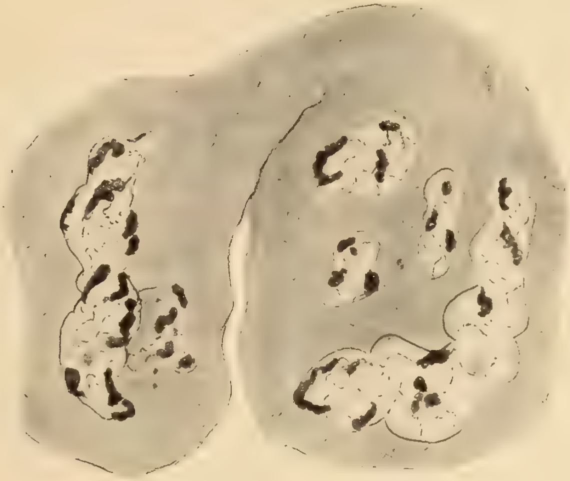
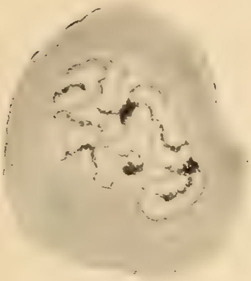


Fig. 3.

FIG. 4.





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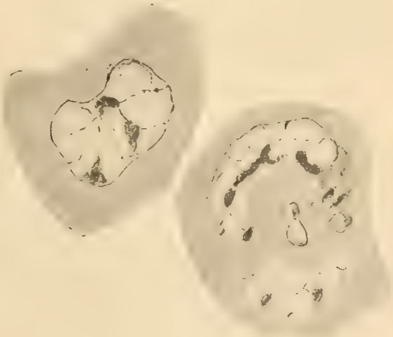

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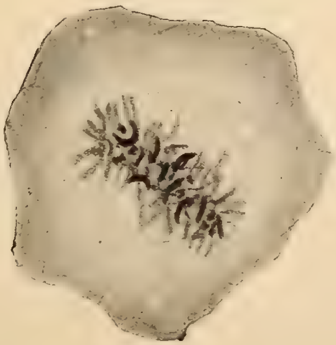

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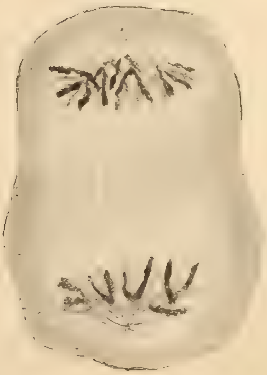


FIG. 8.

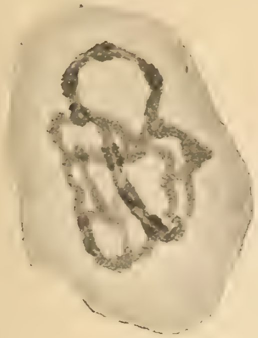


Fig. 9.

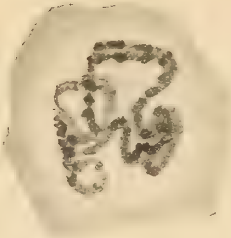


Fig. 10.

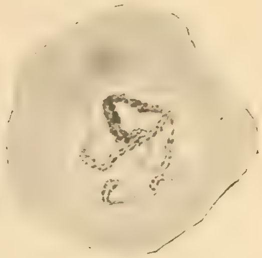
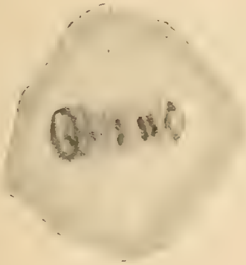


Fig. 11.



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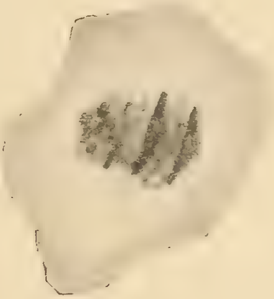


Fig. 13.

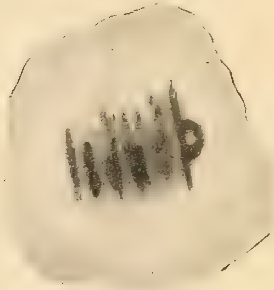


Fig. 14.

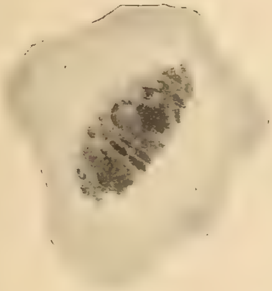
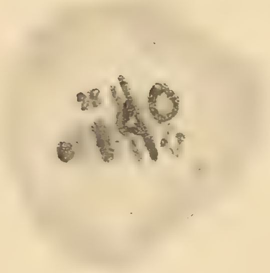


Fig. 15.



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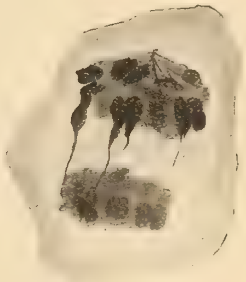

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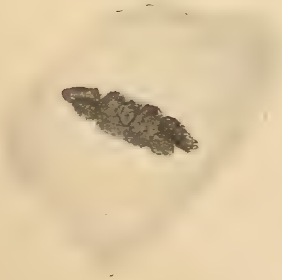
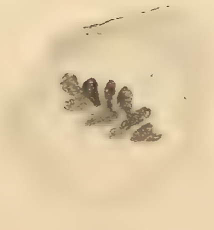


Fig. 18.



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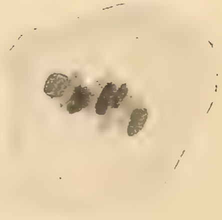

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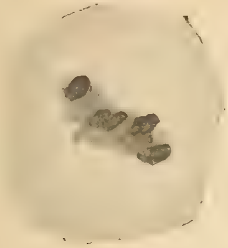

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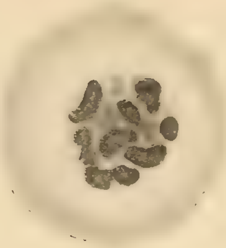

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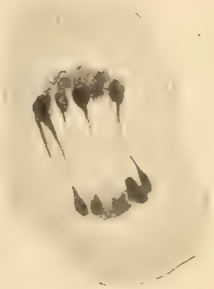


FIG. 23.

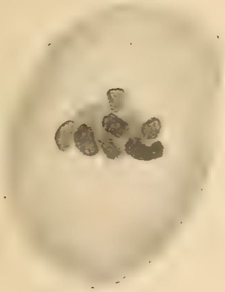
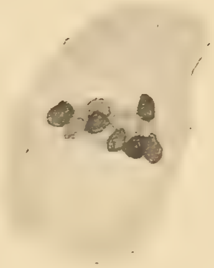


FIG. 24.



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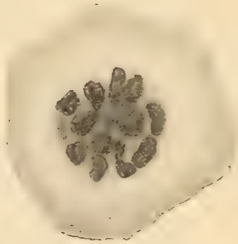

FIG. 26.

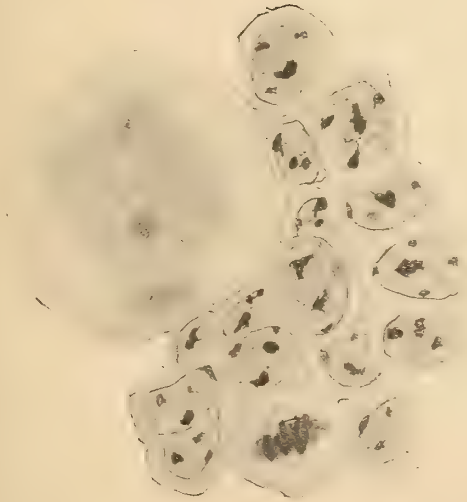

FIG. 27.

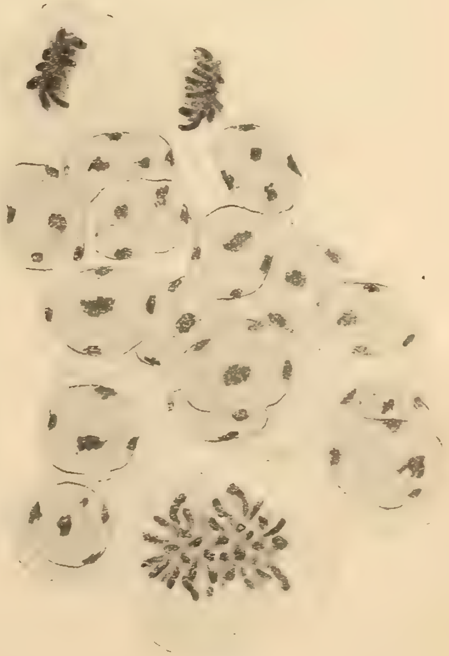

FIG. 28.

PLATE 9.

FIGS. 9 and 10.—Prophases of division in cells of bone-marrow of guinea-pig, showing splitting of the so-called spireme thread.

FIG. 11.—Similar stage in the first maiotic prophase of division in the testis of guinea-pig.

FIGS. 12 and 13.—First maiotic (heterotype) division figures from the bone-marrow of guinea-pig.

FIG. 14.—Ditto from bone-marrow of rat.

FIG. 15.—Division figure from bone-marrow of guinea-pig.

FIG. 16.—First maiotic division figure from testis of guinea-pig.

FIG. 17.—Diaster stage from cell in bone-marrow of guinea-pig. Some of the chromosomes are longitudinally split.

FIGS. 18, 19 and 20.—Second maiotic (homotype) division figures from bone-marrow of guinea-pig.

PLATE 10.

FIG. 21.—Ditto from germinal area of lymphatic gland of guinea-pig.

FIG. 22.—View of equatorial plane of a division figure similar to those shown in figs. 18 to 21.

FIG. 23.—Diaster stage of a similar figure. Bone-marrow, guinea-pig. (Compare with fig. 8.)

FIGS. 24, 25 and 26.—Second maiotic division figures from testis of guinea-pig.

FIG. 27.—Testis of embryo guinea-pig. A large male ovum and a homotype division figure among the cells which will form the foot-cells.

FIG. 28.—Ditto, showing somatic division figures.

The Action on Bacteria of Electrical Discharges of High Potential and Rapid Frequency.

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(Communicated by Professor* J. Rose Bradford, M.D., F.R.S. Received March 30,—Read May 3, 1906.)

(From the Laboratories of the Middlesex Hospital.)

In the course of an investigation into the action of "high frequency" electrical discharges as they are used in the practice of medicine, we have inquired more particularly into the action of such discharges on bacteria exposed to their influence under various experimental conditions.

I. GENERAL PROCEDURE IN EXPERIMENTS, AND ELECTRICAL APPARATUS USED.

The bacteria used for the purposes of our experiments have included the following species:—

B. anthracis, *B. diphtheriæ*, *B. typhosus*, *B. coli communis*, *B. dysenteriæ* (Shiga), *B. pyocyaneus*, and *Micrococcus pyogenes aureus*, together with some saprophytic species, *B. megaterium*, *B. prodigiosus*, and *Micrococcus agilis*.

In our experiments the bacteria have been exposed to the action of the discharges under the following conditions:—

(1) The discharges have been sprayed on to the surface of distilled water, tap water, and normal saline solution in which the bacteria were suspended; and with these circumstances the current has been discharged in different atmospheres above the bacterial emulsion, experiments having been carried out in common air, hydrogen, nitrogen, carbon dioxide and carbon monoxide.

(2) The discharges have been sprayed directly on to the surface of cultures of the several bacteria whilst growing on nutrient-agar and various other solid culture media.

(3) The discharges have been sprayed directly on to the surface of thin films of the bacteria spread out on small slabs of plaster of paris.

And, in addition, we have tested the action of rapidly oscillating electrical currents of high potential when passed directly through bacterial emulsions without interruption by discharge above the surface of the fluid. The experimental conditions included under (2) and (3) above were soon found to be unsatisfactory, inasmuch as a high degree of heat was quickly concentrated in the solid material on which the bacteria were exposed, and it was impossible to appreciate what other kind of influence, if any, the electrical discharge might exert. In what follows we shall refer, therefore, only to the results of experiments carried out under the conditions defined in (1), referring incidentally to the question of the possible influence of the passage through the emulsion of rapidly oscillating currents of high potential without interruption by discharge.

The Electrical Apparatus Used.

In some experiments the oscillatory discharge of high potential obtained at the terminals of the secondary coil of the Ruhmkoff apparatus was transformed by means of a spark-gap, Leyden jars, and resonating Tesla coil into an oscillatory discharge of high potential and extremely rapid frequency.

In the majority of our experiments, however, the apparatus was of different arrangement. The current obtained from the electric lighting main was transformed into an oscillatory current of very high potential by means of an alternator (connected with a transformer) which was driven by a motor. Wires from the alternator were connected with the terminals of a spark-gap and a large condenser immersed in oil. With this form of apparatus the transformation of the current is closely analogous with that occurring when the first mentioned form of apparatus is used.

A third set of experiments were carried out with a Ruhmkoff coil which gave a 12-inch spark; these last experiments being for the purpose of comparing our other results with those which might be obtained by the use of electrical discharges of less rapid frequency, but such as were known to produce under certain circumstances distinct physiological reactions.

In our earlier experiments the action of the discharges was tested by spraying the current from a platinum wire brush on to the surface of either distilled water or normal saline solution containing various bacteria.

The emulsion of bacteria in the test-tube was exposed to the action of discharges sprayed on to them through common air in one or other of the following ways:—

(*a*) The current was discharged from the points of a platinum brush formed by a disc of platinum, 1·5 cm. in diameter, to which were attached nine short lengths of platinum wire; the disc was suspended with its surface parallel to the surface of the bacterial emulsion, the ends of the wire brush being between 2 and 5 cm. above the fluid. In one or two experiments a different form of brush, made by twisting together some lengths of platinum wire, was used, but the resulting discharge was very much less energetic than when the other form of brush was used.

The current being discharged over the emulsion from the brush, it was conducted away by means of a short length of platinum wire which was sealed through the bottom of the test-tube and dipped into a layer of mercury at the bottom of a beaker of water in which the test-tube was suspended for cooling purposes, the level of the water in the beaker being kept well above the level of the bacterial emulsion in the test-tube. From the layer of mercury in the beaker an "earth wire" passed to a gas supply pipe.

In some of the experiments carried out with this arrangement of apparatus the cooling beaker was not used, and the electrical current was allowed to escape into the air as a brush discharge from the platinum wire which passed through the bottom of the test-tube.

(*b*) In another set of experiments there was a layer of mercury at the bottom of the bacterial emulsion in the test-tube, but no wire passing from the tube. In these experiments the results were not satisfactory, for the current appeared to diffuse in various directions after its discharge, and apparently but a small proportion of it passed through the emulsion.

(*c*) In another type of tube which was used, a cap of tin foil encapsuling the lower end of the tube was substituted for the length of platinum wire passing through the bottom of the tube; a few turns of copper wire were wound round this metal cap, and the efferent end of the wire was attached to a gas pipe. This type of tube also was found to give unsatisfactory results; when using it the current seemed to discharge itself chiefly towards the side of the glass tube at the upper level of the emulsion and then to pass down the side of the tube to the metal cap without passing through the emulsion itself.

In the experiments detailed in what follows it is to be understood that the arrangement for conducting the current after its discharge above the emulsion was that described under (*a*), and, unless the contrary is stated, the platinum disc brush was used. The arrangements described under (*b*) and (*c*) are mentioned for the reason that the apparently different course of the current subsequent to discharge which was observed with their use served to confirm our opinion that with the use of arrangement (*a*) the current after its discharge did actually pass directly through the emulsion.

For the course of these experiments it seemed probable that the bacterial emulsion was subjected to the influence of two factors: first there was the action of the discharge on the surface from the platinum brush, and then there was the action of such part of the current, if any, which subsequently passed through the fluid.

And as it appeared to us that the passage of a maximum portion of the current through the emulsion was obtained with the first described arrangement (*a*), all the crucial experiments detailed in this paper were carried out with the kind of tube described, fresh distilled water being usually used for making the emulsion. Before proceeding it will be as well to give the reasons for our assumption that with this type of tube the current when discharged on to the surface is subsequently conducted directly through the fluid.

(1) When using the type of tube described the visible direction of the discharge is directly on to the surface of the fluid, and not towards the sides of the tube, as frequently is the case when the other arrangements are used.

(2) On raising the platinum wire which passes through the bottom of the tube from the mercury in which it is usually immersed a brilliant discharge breaks from its lower end.

(3) If the wire which passes from the mercury in the beaker to the gas pipe is removed for about half an inch from the pipe a brilliant discharge across the gap breaks.

(4) If tap water was used instead of distilled water, or even if distilled water which had been allowed to stand exposed to the air for a few days was used instead of freshly distilled water, a considerable portion of the current, instead of passing by the direct path through the fluid, appeared to pass from the edge of the platinum disc to the side of the test-tube, and thence through the water in the containing-beaker to the earth wire; whereas, when freshly distilled water was used, there was no indication of discharge towards the side of the tube, but the discharged current appeared to impinge directly on to the surface of the fluid, and apparently passed through it to the wire at the bottom of the tube.

(5) Unless special means were adopted for keeping the tube cool, a considerable and rapid rise in the temperature of the emulsion occurred.

It is true, however, that an alternative route was possible theoretically; that is to say, it is conceivable that after its discharge the current might be deflected at a right angle along the surface of the fluid, and then pass down between the side of the tube and the contained emulsion to the wire at the bottom of the tube. But there was no indication that this route was followed, and what we actually observed appeared to us to justify the conclusion at which we arrived as to the direct passage of the current through the emulsion.

In testing the effect of the discharges upon bacteria, the following was the procedure followed:—The bacterial emulsion was prepared by rubbing up two loopfuls of growth, generally taken from a 48-hours-old culture on agar, with 10 c.c. of distilled water. When a uniform emulsion had been made, a loopful of it was streaked out on each of two sloped nutrient agar tubes.

The emulsion was then subjected to the action of the discharge for the required time, at the end of which two agar tubes were each inoculated with a loopful of the treated emulsion. All the agar tubes were then incubated side by side at a temperature suitable to the growth of the particular organism under experiment, and any differences in growth on the experimental and control tubes were noted from time to time. At the conclusion of an experiment any necessary chemical examination of the emulsion was made immediately after the agar tubes had been inoculated.

II.—THE GERMICIDAL ACTION OF THE DISCHARGE WHEN SPRAYED THROUGH AN ATMOSPHERE OF COMMON AIR.

Preliminary experiments, in which the bacterial emulsions contained in tubes of the type described under (*a*) were exposed to the action of the discharge of oscillatory currents of high potential and rapid frequency, sprayed on to the surface of the fluid through an atmosphere of common air, resulted in rapid destruction of the organisms. And we found that the discharge of the current on the surface of the emulsion was followed by the development of a considerable degree of acidity in the fluid, due chiefly, at any rate, to the presence of nitrous and nitric acids in solution.

Tables I and II give the results obtained in two sets of such experiments, in all of which precaution was taken to keep the bacterial emulsion at a temperature which could not by itself possibly affect the bacteria unfavourably, being below 30° C. in every case.

In the experiments detailed in Table I the current was transformed from the electric lighting main; the experiments of Table II were carried out with a powerful induction coil which gave a 12-inch spark, and the platinum brush was connected up with one terminal of the secondary coil, whilst the other terminal was connected with the layer of mercury at the bottom of the beaker in which the tube containing the bacterial emulsion was suspended. In every case, except in the experiments of Series 4 and 5 of Table II, the ends of the platinum brush were 2·5 cm. above the surface of the emulsion.

From the results given it will be seen that, except for the two sporing species, *B. anthracis* and *B. megaterium*, the bacteria tested were destroyed after comparatively short exposure to the discharge, when the ends of the brush were suspended at a distance of 2·5 cm. above the surface of the fluid, whilst in the few experiments included in Series 4 and 5 of Table II, when the brush was suspended 5 cm. above the surface of the fluid, a non-sporing organism, *B. coli communis*, escaped complete destruction, although some inhibition of subsequent growth was observed. In experiments with *B. anthracis* our results were not quite consistent; in the experiment of

Series 1, on Table I, this bacillus survived exposure to the discharge for 15 minutes, with a development of acidity in the emulsion equivalent to 0·19 per cent. of nitric acid, whilst in the experiment of Series 2 of the same table the bacillus was destroyed after exposure to the discharge for only 10 minutes, with resulting acidity of the emulsion equivalent to 0·14 per cent. of nitric acid. But an occasional inconsistency of results is only what may be expected in experiments of this kind, in which the primary factor is a form of electrical force which cannot be regulated with accuracy, and does not affect the main points at issue.

Table I.

Species.	Duration of exposure to the discharge.	Current at 100 volts supplied to the motor driving alternator.	Acidity (calculated as nitric acid per c.c.) of bacterial emulsion at end of experiment.	Percentage of acid in emulsion after experiment (calculated as nitric acid).	Result of experiment.
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 1.	mins.	ampères.	gramme.		
B. anthracis......	15	7	0·0019	0·19	Some growth, but marked inhibition
B. typhosus	15	7	0·0024	0·24	0
B. dysenteriæ ...	15	7	0·0023	0·23	0
B. coli communis	15	7	0·0021	0·21	0
B. pyocyaneus ...	15	7	0·0023	0·23	0
M. agilis	15	7	0·002	0·2	0
Series 2.					
B. anthracis	10	6·75	0·0014	0·14	0
B. typhosus	10	6·75	0·0013	0·13	0
B. dysenteriæ ...	10	6·75	0·0014	0·14	0
B. coli communis	10	6·75	0·0016	0·16	0
B. pyocyaneus ...	10	6·75	0·0014	0·14	0
M. agilis	10	6·75	0·0014	0·14	0
Series 3.					
B. anthracis......	10	6	0·0008	0·08	+
B. typhosus	10	6	0·0011	0·11	0
B. dysenteriæ ...	10	6	0·0008	0·08	0
B. coli communis	10	6	0·0012	0·12	0
B. pyocyaneus ...	7·5	9	0·0009	0·09	0
M. agilis	15	9	0·0025	0·25	0
Series 4.					
B. typhosus	7	5	—	—	0
B. coli communis	7	5	—	—	0
B. pyocyaneus ...	7	5	—	—	0
B. megaterium ...	7	5	—	—	Some growth, but marked inhibition
B. prodigiosus ...	7	5	—	—	0

Table II.

Species.	Duration of exposure to the discharge.	Current and voltage (primary coil).	Acidity (calculated as nitric acid) of bacterial emulsion at end of experiment.	Percentage of acid in emulsion at end of experiment (calculated as nitric acid).	Result of experiment.
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 1.	mins.		gramme.		
B. anthracis......	20	4 ampères 74 volts	0·0004	0·04	+
B. typhosus	20	„	0·0005	0·05	0
B. dysenteriæ ...	20	„	0·0001	0·01	0
B. coli communis	20	„	0·0009	0·09	0
B. pyocyaneus ...	20	„	0·0004	0·04	0
M. agilis	20	„	0·0009	0·09	0
Series 2.					
B. anthracis......	15	6 ampères 80 volts	0·0005	0·05	+
B. typhosus	15	„	0·0006	0·06	0
B. dysenteriæ ...	15	„	0·0007	0·07	0
B. coli communis	15	„	0·0004	0·04	0
B. pyocyaneus ...	15	„	0·0005	0·05	0
M. agilis	15	„	0·001	0·1	0
Series 3.					
B. anthracis......	10	6 ampères 84 volts	0·0005	0·05	Some growth, but marked inhibition
B. typhosus	10	„	—	—	0
B. dysenteriæ ...	10	„	—	—	0
B. coli communis	10	„	0·0008	0·08	0
B. pyocyaneus ...	10	„	0·0006	0·06	0
M. agilis	10	„	—	—	0
Distance of Platinum Brush above Surface of Fluid, 5 cm.					
Series 4.					
B. coli communis	10	6 ampères 80 volts	—	—	Some growth
B. pyocyaneus ...	12	„	—	—	0
„ ...	10	„	—	—	0
M. agilis	6	„	—	—	0
Series 5.					
B. coli communis	5	6 ampères 80 volts	—	—	Some growth
B. pyocyaneus ...	5	„	—	—	0
„ ...	5	„	—	—	0
Distance of Platinum Brush above Surface of Fluid, 2·5 cm.					
Series 6.					
B. anthracis......	10	5·5 ampères 70 volts	—	—	+
B. typhosus	10	„	—	—	0
B. coli communis	10	„	—	—	0
B. pyocyaneus ...	10	„	—	—	0
B. prodigiosus ...	7	„	—	—	0

Having thus obtained definite evidence of the germicidal effect of the electrical discharge under the given experimental conditions, we had next to consider the respective influence of several possible factors in the production of the results which we had observed.

These possible factors, as they presented themselves to us, may be enumerated as follows:—

(1) What may be termed the specific physical action of the current, or of its discharge, on the bacteria in the emulsion;

(2) The action of light rays resulting from the discharge;

(3) The action of heat rays resulting from the discharge; and

(4) The action of certain chemical substances which are formed in the air as the result of the discharge, and are then taken up in solution by the water in which the bacteria are suspended.

III.—AS TO THE GERMICIDAL ACTION, IF ANY, OF THE ELECTRICAL CURRENT, OR OF ITS DISCHARGE.

We met with considerable difficulties in our endeavour to determine what part, if any, in the production of the germicidal effects which had been obtained in the previously mentioned experiments could be attributed to the action on the bacteria of the electric current itself, as apart from secondary factors depending upon the discharge—the action of heat and light rays and of chemical substances formed in the neighbourhood of the emulsion and taken up by it. For, whilst it was easy to eliminate experimentally any injurious action which might be exerted directly by the heat rays and to test by itself the possible action of the light rays, we found the greatest difficulty in devising means whereby the bacteria could be subjected to the action of the electric current without at the same time introducing the possibility of fallacy arising from chemical decomposition with the formation of germicidal substances.

We first tested the action of the oscillatory current when passed directly through the bacterial emulsion without interruption by discharge above the surface. In these experiments a flat platinum disc, which was immersed just below the surface of the emulsion, was substituted for the platinum brush. In some experiments the emulsion was contained in a test-tube, with arrangement for conducting the current away as described under (*a*) above, but in the majority of the experiments there was the addition of a layer of mercury in the tube under the emulsion and covering the projecting upper end of the wire which was sealed through the bottom of the tube.

The latter modification appeared to be the more suitable for testing the full action of the current on the bacterial emulsion, and we found that when efficient means were adopted for keeping the temperature of the emulsion below the point at which the germicidal effect of heat would come into play, and when there was no great amount of "sparking" from the wire to the platinum disc, the mere passage of the current through the emulsion for periods of time corresponding to those prevailing in the previous experiments had no obvious effect on the bacteria. In certain experiments of this kind, in which the bacteria were destroyed, the emulsion was found to contain at the end of the experiment definite quantities of chemical germicidal substances which had been formed by excessive sparking just above the level of the emulsion from the wire which was connected with the immersed platinum disc.

If special precautions for keeping the emulsion cool were not taken, the heating effect of the passage of the current through the emulsion was very marked in these experiments with the immersed disc, the temperature rising rapidly to 55° C. or higher.

The following experiments with somewhat prolonged passage of the current through the emulsion may be specially referred to. The current was applied in each experiment for six successive periods of 10 minutes each, with intervals of five minutes between, and the tube containing the emulsion was kept surrounded by melting ice. Under these conditions the temperature of the emulsion never rose above 32° C., a degree of heat which, for the time duration of the experiments, would not have any injurious effect upon either of the species tested.

Experiment 1.—A thick, deep red emulsion of *B. prodigiosus* in distilled water was exposed to the action of the current. A certain amount of sparking occurred just above the surface of the emulsion from the wire which was connected with the terminal platinum disc. The red colour of the emulsion was quickly discharged, and the bacteria were all killed at the end of the experiment. The emulsion had become strongly acid, 1 c.c. of it containing 0·1 c.c. of N/40 acid, equivalent to 0·016 per cent. of nitric acid.

In a control experiment, carried out without immersion of the tube in melting ice, the temperature of the emulsion had risen to 65° C. at the end of the 60 minutes' exposure.

Experiment 2.—The organism tested was *B. pyocyaneus* in an emulsion with normal saline solution, and a constant stream of hydrogen was passed through the fluid. In this instance, again, the bacteria were all destroyed at the end of the experiment, and the emulsion was found to contain in solution some substance which was capable of liberating iodine from iodide of potassium.

Experiment 3.—An emulsion of *B. pyocyaneus* with normal saline solution was again used; hydrogen was passed through the tube above the level of the emulsion so as to exclude atmospheric air without causing the constant splashing of the fluid which had occurred in the last experiment. After 60 minutes' exposure, as in the other experiments, to the action of the current, the bacteria were found to be unaffected, the emulsion yielding equally free growth before and after treatment.

A large number of experiments in which the discharge was sprayed on to the emulsion through an atmosphere of some pure gas were also carried out, with appropriate arrangements for the conduction of the current through the emulsion. These experiments will be referred to in detail after the influence of the nitrous compounds which are formed when the discharge occurs in common air have been considered, and meanwhile it may be said that the result of such further experiments was in confirmation of the conclusion at which we had already arrived, that is to say, it appeared as if the discharge and subsequent passage of the current through the emulsion had, of itself, no injurious influence on the bacteria under the time conditions of our experiments.

IV.—THE POSSIBLE ACTION OF LIGHT RAYS RESULTING FROM THE ELECTRICAL DISCHARGE.

Experiments were carried out in order to ascertain whether the light rays resulting from the electrical discharge might have had any definite influence in the production of the germicidal effects which were observed in our previous experiments. And in the result it may be said that we were able to exclude any such factor as operative under the time conditions of our experiments.

The following experiment bearing on this point may be mentioned. Tubes of melted nutrient agar were cooled down to 41° C., and inoculated severally from cultures of the bacteria with which we had previously experimented; the medium was then poured out into Petri dishes, and allowed to set. The covers of the Petri dishes were thickly coated with black varnish, except for a small circular area which was left unprotected. The Petri dishes were then closely exposed for 60 minutes to the action of light rays from discharges such as those which had been employed in the other experiments, the dishes being so arranged that the maximum effect of the rays would be exercised on the area of inoculated agar immediately underlying the unprotected part of the cover. After exposure the dishes were incubated at temperatures suitable to growth of the respective bacteria, and in every case good growth was obtained over the whole surface of the medium, there being no indication of inhibition of growth in those portions which had been specially exposed to the action of the light rays.

V.—THE INFLUENCE OF HEAT RAYS RESULTING FROM THE ELECTRICAL DISCHARGE.

In all our experiments, unless the contrary is expressly stated, means were adopted to prevent the temperature of the bacterial emulsion rising to a point at which the direct germicidal action of heat would come into play. The highest temperature reached, when limiting precautions were adopted, was about 32° C., which was recorded in some experiments which have already been referred to, when the terminal platinum disc was actually immersed in the emulsion; in experiments in which the discharge was sprayed from a platinum brush suspended above the surface of the fluid the maximum temperature reached was usually several degrees below this point.

In order to satisfy ourselves that we were experimenting under safe temperature conditions we tested the resistance to heat of some of the cultures of bacteria which were used in our experiments. We ascertained that the culture of *B. prodigiosus* survived a temperature of 45° C. with an exposure of 60 minutes, but was destroyed by an exposure to 50° C. for 30 minutes; *B. pyocyaneus* survived an exposure to a temperature of 50° C. for 45 minutes, but was killed at the same temperature with an exposure of 60 minutes; the cultures of *B. coli communis* and *Micrococcus pyogenes aureus* were both unaffected by 60 minutes' exposure at a temperature of 50° C.

The results of these tests with regard to temperature and duration of exposure are tabulated below:—

	40° C.	45° C.			50° C.		
	60 mins.	39 mins.	45 mins.	60 mins.	30 mins.	45 mins.	60 mins.
B. prodigiosus	+	+	+	+	0	0	0
B. pyocyaneus	+	+	+	+	+	+	0
B. coli communis ...	+	+	+	+	+	+	+
M. pyogenes aureus	+	+	+	+	+	+	+

But, whilst we were able to eliminate the direct germicidal effect of heat in considering the results of our experiments, it was clear that under certain conditions the germicidal action of the nitrous and nitric compounds and the hydrogen peroxide which were formed as the result of the discharge, and taken into solution in the bacterial emulsion, would be considerably enhanced by a rise of temperature which would not by itself have any direct action on the bacteria; and it is to the varying degree of germicidal energy of these oxidising substances at temperatures between about 15° C. and 35° C. that apparent discrepancies in some of our results must be partly attributed. For with variation in the amplitude of the electric current, which frequently occurred in the course of our experiments, it was not possible to keep the temperature of the emulsions at a constant point; the most that we succeeded in doing was to ensure by cooling arrangements that the temperature did not rise to a point at which the direct germicidal effect of heat would come into action.

VI.—THE INFLUENCE, ON BACTERIA SUSPENDED IN WATER, OF NITROUS AND NITRIC ACIDS RESULTING FROM THE ACTION OF THE ELECTRICAL DISCHARGE ON THE AIR AND TAKEN UP IN SOLUTION.

We next proceeded to test the germicidal action of sterile distilled water which had been exposed to discharges from the high-frequency apparatus whilst contained in tubes under conditions similar to those which prevailed in the experiments detailed in Table I.

On adding iodide of potassium to distilled water which has been exposed to the action of the discharge for a few minutes, a considerable amount of iodine is set free, and further tests show the presence of nitrous and nitric acids in solution.

The immediate products of the action of the discharge on ordinary air probably include ozone, hydrogen peroxide, nitrogen peroxide, and perhaps other oxides of nitrogen. A certain amount of the substances thus formed are taken up in solution by the water, and the ozone and hydrogen peroxide are probably rapidly decomposed by reaction with the other products present, nitric oxide being converted to nitrogen peroxide, and nitrous to nitric acid.

The nitrogen peroxide which is formed reacts with water to form nitrous and nitric acids according to the equation

$$2NO_2 + H_2O = HNO_2 + HNO_3.$$

Under the conditions of the experiment a part of the nitrous acid decomposes into nitric acid, nitric oxide, and water,

$$3HNO_2 = HNO_3 + 2NO + H_2O,$$

and the nitric oxide would probably again be converted into nitrogen peroxide and nitrous and nitric acids.

Our experiments were carried out as follows:—After each tube had been exposed to the action of the discharge for a given time the resulting acidity was estimated in terms of nitric acid, and the germicidal action of the solution was tested on a single species of bacterium. For the latter purpose two loopfuls of growth from a culture on agar were rubbed up into an emulsion with the acid solution, and subcultures were made after the organism had been exposed to the action of the acid solution for periods of 15, 30, and 60 minutes. The inoculated tubes were then incubated for 96 hours at an appropriate temperature, and the amount of growth, if any, compared with that obtained from similar subcultures made from similar emulsions in distilled water.

The results of these experiments are given on Table III:—

Table III.

Species.	Duration of the exposure of water to the discharge.	Current at 100 volts supplied to the motor driving the alternator.	Acidity (calculated as nitric acid per cubic centimetre) of solution at end of experiment.	Percentage of acid in emulsion after experiment (calculated as nitric acid).	Result of exposure of bacteria to acid solution for times as below. + = growth obtained. 0 = no growth.		
					15 mins.	30 mins.	60 mins.
Series 1.							
	mins.	ampères.					
B. typhosus	30	6	0·0022	0·22	0	0	0
B. coli communis ..	30	6	0·0028	0·28	0	0	0
B. prodigiosus ...	30	6	0·0033	0·33	0	0	0
Series 2.							
B. anthracis	15	6	0·001	0·1	+	+	
B. typhosus	15	6	0·0007	0·07	0	0	
B. coli communis ..	15	6	0·0011	0·11	0	0	
B. diphtheriæ......	15	6	0·0008	0·08	0	0	
B. pyocyaneus ...	15	6	0·0013	0·13	0	0	
B. prodigiosus ...	15	6	0·00097	0·097	0	0	

From the results shown in Table III it will be seen that, except as regards the tube in which a sporing culture of *B. anthracis* was treated, the substances taken up from the air by water exposed to the action of the discharge were sufficient to destroy the bacteria subsequently immersed in the acid solution for so short a period as 15 minutes. It will be seen also that, although the tubes used in each series of experiments were exposed to the action of the discharge under conditions as nearly equal as the apparatus and current at our disposal would allow, there was considerable variation in the resulting degree of acidity, as had occurred also in the experiments detailed in Tables I and II. In short, it soon became obvious that we were utilising forces which were not under nice control, and that our results must be taken as being comparative in only an approximate degree.

The extent to which discrepancy in the degree of acid produced occurred, under conditions as nearly equal with regard to current as we could obtain, is shown in the following tabulation of the results of the acidimetry of the contents of each tube after exposure. For purposes of comparison the experiments of Tables I and III may be considered together, as having been carried out with the same apparatus, whilst in the experiments of Table II a different arrangement of electrical apparatus was used, and one which was less suitable to the purposes of our experiments (see p. 66).

Acidimetry Table.

	Current at 100 volts supplied to motor driving the alternator.	Duration of exposure to discharge in mins.	Number of tubes used.	Percentage average acidity, calculated as nitric acid.	Percentage acidity of contents of each tube, calculated as nitric acid.
Table I.					
Series 1.........	7 ampères	15	6	0·216	0·24, 0·23, 0·23, 0·21, 0·2, 0·19
Series 2.........	6·75 „	10	6	0·141	0·16, 0·14, 0·14, 0·14, 0·14, 0·13
Series 3.........	6 „	10	4	0·097	0·12, 0·11, 0·08, 0·08
„	9 „	15	1	—	0·25
„	9 „	7·5	1	—	0·09
Table III.					
Series 1.........	6 „	30	3	0·276	0·33, 0·28, 0·22
Series 2.........	6 „	15	6	0·099	0·13, 0·11, 0·1, 0·097, 0·08, 0·07
	Current and voltage (primary coil).				
Table II.					
Series 1.........	4 ampères 74 volts	20	6	0·053	0·09, 0·09, 0·05, 0·04, 0·04, 0·01
Series 2.........	6 ampères 80 volts	15	6	0·061	0·1, 0·07, 0·06, 0·05, 0·05, 0·04
Series 3.........	6 ampères 84 volts	10	3	0·063	0·08, 0·06, 0·05

We are unable to explain satisfactorily the unequal formation of acid in tubes of distilled water, or of bacterial emulsion, which have been submitted to apparently the same treatment in the course of each separate series of experiments, we can only assume that it is a result of considerable variation in the intensity of the current during the progress of an experiment. The results obtained in the experiments of Table III show, at any rate, that a natural solution of the products of the action of the discharge on common air is sufficient when these products are present in such quantity that the acidity of the fluid is equivalent to 0·07 per cent. of nitric acid to destroy a non-sporing bacillus, such as *B. typhosus*, after it has been exposed to the action of the solution for 15 minutes. And we take it that in the cases in which a bacterial emulsion after sterilisation by exposure to the discharge is found to present an acidity in excess of 0·07 per cent. the sterilisation may be fairly attributed mainly to these chemical products, in the absence of any indication that other actively germicidal influences have been at work.

We may, however, point out that the experiments of Table III are not absolutely comparable with those of Table I, since in the latter set of experiments the quantity of germicidal substances in solution gradually increased up to the end of the exposure, when the estimation gives only the total acidity at the end of the experiment, whereas in the experiments of Table III the bacteria were immersed in a solution of the maximum acidity for the full period of the experiment.

On the other hand, the germicidal action of such substances as are present is probably exercised more energetically under the conditions prevailing in the experiments of Table I, when the bacteria are immersed in the fluid during the actual formation and solution of the nitrous and nitric bodies and are so exposed to the action of these substances whilst still in a nascent condition, than it is under the conditions prevailing in the experiments of Table III, when the bacteria are immersed in a fluid in which the chemical changes are for the most part finished, except for the comparatively slow oxidation of nitrous to nitric acid. Moreover, in the experiments of Table I there would be a continuous molecular disturbance of the fluid which would probably assist the action of chemical germicides.

Although other substances are formed in the air, and doubtless taken up in solution by the water, in addition to the nitrous and nitric compounds, it was to these latter that we attributed chiefly the germicidal action, and it was these that we considered as the causes of the acidity of the solution. We next prepared artificial mixtures of nitrous and nitric acid with a total acidity approximating to that obtained in some of our experiments, and then tested the germicidal action of the solutions. Our solutions were prepared by dissolving potassium nitrite in water and adding certain quantities of nitric acid.

The actual composition of the several solutions was as follows:—

Solution.	Weight of potassium nitrite in 1 c.c.	Equivalent weight of nitrous acid in 1 c.c.	Weight of nitric acid in 1 c.c.
	gramme.	gramme.	gramme.
a	0·00074	0·00041	0·0005
b	0·00148	0·00082	0·001
c	0·00222	0·00123	0·0015
d	0·00296	0·00164	0·002
e	0·0037	0·00205	0·0025

Assuming that all the available nitrous acid is liberated by the nitric acid added, the composition of each solution at the time of carrying out the experiment would be—

Solution.	Weight of nitrous acid in 1 c.c.	Weight of nitric acid in 1 c.c.	Weight of potassium nitrate in 1 c.c.
	gramme.	gramme.	gramme.
a	0·00041	0·00009	0·00088
b	0·00082	0·00018	0·00176
c	0·00123	0·00027	0·00264
d	0·00164	0·00036	0·00352
e	0·00205	0·00045	0·0044

But whatever the interaction between the nitrite and the nitric acid may be, one may assume that the acidity of the respective solutions was 0·05, 0·1, 0·15, 0·2 and 0·25 per cent. respectively, calculated as nitric acid.

Nitrous acid being unstable, and decomposing readily at ordinary temperatures into nitric acid, nitric oxide, and water, we carried out two sets of experiments, one at a temperature of 0° C., the other at a temperature of 15° C.

Immediately after they had been prepared the respective solutions were inoculated with two loopfuls of the several bateria tested: loopfuls of the acid emulsion were taken immediately after the bacteria had been introduced ("momentary" exposure), after an interval of 15 minutes, and after an interval of 30 minutes nutrient agar tubes were inoculated with the loopfuls and incubated side by side with control tubes.

The results of the experiments are given in Table IV.

Table IV.

Acidity of solution estimated in terms of nitric acid.	Temperature at which experiment was carried out.	*B. anthracis* (sporing culture).			*B. typhosus.*			*B. pyocyaneus.*			*B. prodigiosus.*		
		Duration of exposure.			Duration of exposure.			Duration of exposure.			Duration of exposure.		
		"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.	"Momentary."	15 minutes.	30 minutes.
	°C.												
a. 0·05 p.c. ...	0	+	+	+	+	0	0	+	0	0	+	Very scanty	0
	15	+	0	0	0	0	0	+	0	0	+	0	0
b. 0·1 p.c. ...	0	+	+	+	+	0	0	0	0	0	+	0	0
	15	+	0	0	0	0	0	0	0	0	+	0	0
c. 0·15 p.c. ...	0	+	+	+	+	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0
d. 0·2 p.c. ...	0	+	+	+	Very scanty	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0
e. 0·25 p.c. ...	0	+	+	0	0	0	0	0	0	0	0	0	0
	15	+	0	0	0	0	0	0	0	0	0	0	0

VII.—AS TO THE ACTION OF OZONE AND HYDROGEN PEROXIDE FORMED IN THE AIR AS THE RESULT OF THE ELECTRICAL DISCHARGE.

Ozone and hydrogen peroxide had to be considered amongst the products resulting from electrical discharge in an atmosphere of common air and in the presence of aqueous vapour, for certain quantities of these substances would doubtless be taken up in solution by the bacterial emulsion.

But under the conditions of the experiments, that is to say with a simultaneous formation of nitrous acid, it is, we think, quite certain that any quantity of ozone or hydrogen peroxide which passed into solution would be immediately decomposed. And, therefore, the importance of these two substances in these experiments probably does not lie in any relation to their germicidal action, but rather in the part which they play in the oxidation of the nitrous compounds.

Peroxide of hydrogen, however, became of direct importance in some experiments in which the electrical discharge was effected in an atmosphere of pure hydrogen, for in this case it appeared as if this substance were the active germicidal agent in certain cases which will be referred to in detail further on.

VIII.—RESULTS OF EXPERIMENTS IN WHICH THE ELECTRICAL DISCHARGE WAS SPRAYED THROUGH AN ATMOSPHERE OF VARIOUS PURE GASES ON TO BACTERIA SUSPENDED IN WATER.

The remaining experiments which we have to describe were carried out by spraying the electrical discharge on to the bacterial emulsion through an atmosphere of one or other of the following pure gases:—Hydrogen, carbon dioxide, carbon monoxide, and nitrogen. The object of these experiments was, by discharging the current under conditions such that electrolytic changes were likely to be reduced to a minimum, to observe the action of the discharge and current on the bacteria without the concomitant germicidal action of the nitrous and nitric acids which are formed under the ordinary conditions when the discharges are used in medical practice. In some instances we succeeded in our object, inasmuch as we found that when electrolytic changes were inappreciable, or nearly so, we were able to expose the bacteria to the action of the discharge for periods of 30 and 60 minutes without affecting their subsequent growth in any way. But in many other experiments we found that, even when the discharge occurred in an atmosphere of a pure gas, electrolytic changes resulted after a time, in most cases probably depending upon the rising of water vapour in the tube from the emulsion with the formation of peroxide of hydrogen and possibly other germicidal substances.

Experiments in an Atmosphere of Pure Hydrogen.

The hydrogen used in our experiments was prepared by the action of diluted pure sulphuric acid on pure zinc, the gas being passed successively through solutions of potassium hydroxide, potassium permanganate, and silver nitrate.

The gas thus prepared had no appreciable action on bacteria under the time conditions of our experiments; it was passed continuously for 90 minutes through bacterial emulsions such as those used in our subsequent experiments, and sub-cultures made from the emulsion after the passage showed that the gas had no injurious action.

A few preliminary experiments were carried out with emulsions of *B. pyocyaneus* and *B. prodigiosus*, the current being discharged from a brush of twisted platinum wire suspended in an atmosphere of hydrogen 2·5 cm. above the emulsion. The results are given in Table V:—

Table V.

Species.	Duration of exposure to the discharge.	Current at 100 volts supplied to motor driving the alternator.	Reaction of the emulsion at the end of the exposure.	Result of sub-culture from the emulsion after the exposure.
	mins.	ampères.		
B. pyocyaneus......	30	5·8	Neutral	Good growth, pigment formation normal
	60	5·8	,,	,, ,,
B. prodigiosus......	30	5·0	,,	,, ,,
	60	5·0	,,	,, ,,

In these experiments there was no appreciable change in the reaction of the emulsion after exposure to the discharge; in a set of control experiments carried out with exactly the same arrangement of apparatus, but with the discharge in an atmosphere of common air, the emulsion became strongly acid from the formation of nitrous and nitric acids, and the bacteria were destroyed at the end of the 30 minutes' exposure.

In other experiments, in which the action of the discharge in an atmosphere of hydrogen was tested, distinct germicidal effects were observed in some instances. In these experiments a more energetic discharge was obtained by the use of the platinum disc brush with nine points of platinum wire; in one series of experiments the current was obtained from the alternator, in one other series by means of the Ruhmkoff coil, and the hydrogen was passed continuously through the emulsion during its exposure, with splashing of the emulsion as a consequence. The results are given in Table VI, some of the tests being carried out in duplicate.

It will be seen that in a number of the experiments detailed in Table VI the bacteria were destroyed; with the current from the alternator *B. typhosus* was destroyed after an exposure of 60 minutes, having previously survived an exposure of 30 minutes, and *Micrococcus agilis* was destroyed after an exposure of 30 minutes, whilst all the other bacteria survived. With the use of the current from the Ruhmkoff coil germicidal action was more frequent; *B. typhosus* was destroyed after an exposure of 30 minutes, *B. dysenteriæ* was destroyed after an exposure of 30 minutes in one instance, but yielded a scanty growth in a duplicate experiment; *B. coli communis* was destroyed after an exposure of 30 minutes in one experiment, but survived an exposure of 60 minutes in another; *B. pyocyaneus* was destroyed after an exposure of 30 minutes in duplicated experiments, and *Micrococcus agilis* was destroyed after an exposure of 30 minutes.

Table VI.

Species.	Duration of exposure.	Experiments with current at 100 volts (5 ampères) supplied to motor driving the alternator.		Experiments using Ruhmkoff coil.	
		Reaction of emulsion at end of exposure.	Result.	Reaction of emulsion at end of exposure.	Result.
	mins.				
B. anthracis	30	—	—	Neutral	+
	60	Neutral	+		
	60	,,	+		
B. typhosus	30	,,	+	,,	0
	30	,,	+		
	60	,,	0		
	60	Faintly alkaline	0		
B. dysenteriæ (Shiga)	30	—	—	,,	Scanty growth
	30	—	—	,,	0
B. coli communis	30	Neutral	+	,,	0
	60	—	—	,,	+
B. pyocyaneus	30	Neutral	+	,,	0
	30	,,	+	,,	0
	60	,,	+		
	60	Faintly alkaline	+		
B. prodigiosus	30	Neutral	+		
	30	,,	+		
	60	,,	+		
	60	,,	+		
Micrococcus agilis	30	,,	0	Neutral	0

In two of the experiments the reaction of the emulsion was found to be faintly alkaline at the end of the exposure, one experiment being that in which *B. typhosus* was destroyed after exposure to the current from the alternator for 60 minutes, and the other one of the instances in which *B. pyocyaneus* survived exposure to the same current for 60 minutes. In all the other experiments, whether the current was obtained from the alternator or from the Ruhmkoff coil, the reaction of the emulsion remained practically neutral after the exposure.

In the experiments of Table V the atmosphere of hydrogen was obtained by passing the gas into the tube above the surface of the emulsion; in the other experiments the gas was passed through the emulsion with resulting disturbance of the liquid and increased opportunity for electrolytic changes to occur, and, moreover, in the experiments of Table VI a somewhat more energetic discharge was obtained from the disc brush than from the twisted wire brush used in the earlier experiments. We had, therefore, to consider whether the germicidal effect which had been produced in some of the experiments carried out under the conditions more favourable to electrolytic changes might have been caused by the known possible substances which might be produced by the action of an electrical discharge on hydrogen in the presence of water (hydrogen oxide) vapour.

The substances which theoretically might be thus produced are ozone, nascent oxygen, nascent hydrogen, and hydrogen peroxide, and we proceeded to try whether we could detect either ozone or hydrogen peroxide after discharging the current in an atmosphere of pure hydrogen over water, the discharges being continued for periods of 15, 30, and 60 minutes. In each case peroxide of hydrogen could be detected in the water after exposure to the discharge, and a piece of moist starch iodide paper introduced into the tube at the termination of each time exposure gave no indication of the presence of ozone.

We next carried out two sets of experiments in order to ascertain how far the germicidal effect produced in some of the previous experiments could be attributed to the action of chemical substances, of which hydrogen peroxide was at any rate an important one, taken up in solution by the emulsion.

In each experiment 25 c.c. of distilled water in a test-tube were exposed to the action of the discharge for 60 minutes: in the first set of experiments pure hydrogen was passed through the water in order to expel any dissolved oxygen or nitrogen before exposure to the discharge commenced, and was then passed into the tube above the level of the water for the 60 minutes' exposure; in the second set of experiments a stream of hydrogen was passed continuously through the water during the whole exposure. After exposure to the discharge 5 c.c. of the treated water were titrated with sodium thiosulphate and starch for the quantitative estimation of the peroxide of hydrogen, the presence of which was indicated by the various tests applicable. Quantities of 5 c.c. were then pipetted off into test-tubes, and inoculated respectively with two loopfuls of cultures of *B. anthracis*, *B. coli communis*, and *B. typhosus* from agar tubes. Loopfuls of the bacterial emulsion were transplanted to agar tubes at intervals of 15, 30, and 60 minutes, and incubated in the usual way to test the vitality of the bacteria after their immersion in the solution of hydrogen peroxide.

The results of these two sets of experiments are given in Table VII.

Table VII.

Cultures taken from the bacterial emulsion after	Quantity of peroxide of hydrogen in solution after exposure to the discharge for 60 minutes.					
	I.—One part in 15,000 (approximately).			II.—One part in 10,000.		
	15 mins.	30 mins.	60 mins.	15 mins.	30 mins.	60 mins.
B. anthracis	+	+	+	+	+	+
B. coli communis	+	+	+	+	+	0
B. typhosus	+	+	+	0	0	0

The results of these last experiments showed that when the discharge was sparked on to distilled water through an atmosphere of hydrogen under conditions similar to those prevailing in the experiments of Table V, except that the platinum disc brush was used instead of the twisted wire brush, peroxide of hydrogen was taken up in solution to the amount of 1 in 15,000, and bacteria, which were subsequently immersed in the solution for as long as 60 minutes, were not apparently affected in any way. But when the discharge was sparked on to the water whilst hydrogen was passing continuously through it, under conditions similar to those prevailing in the experiments of Table VI, the amount of hydrogen peroxide taken up in solution increased to 1 in 10,000, and *B. typhosus* and *B. coli communis* were destroyed after immersion in the solution for 15 and 60 minutes respectively. We were unable to detect any change in the chemical composition of the water other than that due to the presence of peroxide of hydrogen, and it was to the presence of this substance that we attributed the germicidal action of the solution.

In comparing the results of the experiments of Tables VI and VII it must be remembered that, as in the similar experiments with nitrous compounds, the germicidal action of peroxide of hydrogen is most active when the substance is in the nascent condition. Thus Bonjean* found that the nascent hydrogen peroxide liberated from calcium peroxide completely sterilised the notoriously foul Seine water in four hours when acting in a dilution of 1 in 14,285, whilst a dilution of 1 in 3445, when obtained by means of a commercial solution of hydrogen peroxide, did not sterilise the same polluted water until it had acted for six hours. In the experiments of Table VI, moreover, it appeared probable that in addition to the action of peroxide of hydrogen there might be another factor at work, for a consideration of the chemical decomposition by which the peroxide was produced suggested the simultaneous liberation of quantities of nascent hydrogen which would doubtless also exercise a powerful germicidal action.

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* 'Comptes Rendus de l'Académie des Sciences,' vol. 140, p. 50, 1905.

Assuming, however, that the germicidal action of peroxide of hydrogen was the main factor concerned, we found that some experiments which we carried out to test the comparative resistance of the bacteria used in the experiments to the action of the peroxide gave results which were roughly in accordance with those detailed in Table VI. We found that sporing cultures of *B. anthracis* were strongly resistant; experiments carried out at temperatures of 0° C., 15° C., and 26° C. with a 1-per-cent. solution of peroxide of hydrogen showed that with 30 minutes' immersion no obvious effect on the bacteria was produced at any of the temperatures, with 60 minutes' immersion no effect was produced at a temperature of 15° C., but there was distinct inhibition of subsequent growth in experiments carried out at 26° C., whilst the bacteria were destroyed after 120 minutes' immersion at all three temperatures. With regard to the non-sporing bacilli, we found that *B. prodigiosus* and *B. pyocyaneus* were more resistant to the peroxide than *B. coli communis*, *B. dysenteriæ*, and *B. typhosus*, the last mentioned being readily destroyed by very dilute solutions, and *B. prodigiosus* exhibiting a marked degree of resistance. In the performance of these experiments it was noted that the rapidity of the decomposition of the peroxide in the solution, and the consequent activity of germicidal action, appeared to depend to some extent upon individual peculiarities in the chemical constitution of the various species used in the tests. The experiments were carried out by introducing definite quantities of cultures of each species from nutrient agar into test-tubes containing the diluted peroxide and kept at the several temperatures. And it was apparent that the rapidity with which the peroxide was decomposed, as measured by the evolution of oxygen in bubbles from the solution, varied widely with different species at the same temperature; for any one species the evolution of gas was more rapid at the higher temperature, with corresponding intensity of germicidal action.

In these experiments to test the germicidal action of solutions of peroxide of hydrogen, a special effect of the peroxide in temporarily inhibiting the formation of pigment by chromogenic bacteria which had been immersed in solutions of it was also noticed. Thus, after an immersion for 60 minutes in a 1 in 500 solution of peroxide of hydrogen kept at a temperature of either 15° C. or 26° C. the amount of subsequent growth of *B. prodigiosus* was in no way affected; but whereas in control cultures of the bacillus which had not been exposed to the action of the peroxide the formation of pigment was obvious from the first appearance of growth, in the cultures from bacilli which had been immersed in the solution the formation of pigment was not apparent until the third or fourth day of incubation. This effect was repeatedly noticed in experiments with this organism, and also with the other chromogenic organism tested, *B. pyocyaneus.*

Experiments in an Atmosphere of Pure Carbon Dioxide.

We next tested the action of the discharges when sprayed on to bacterial emulsions through an atmosphere of pure carbon dioxide, the gas having been prepared by the action of hydrochloric acid on marble and purified by passage through a solution of nitrate of silver.

Experiments which have been recorded by Dr. Charles Slater* showed that it was improbable that under the time conditions of our experiments carbon dioxide itself would have any action on the bacteria; but we first tested the action of the gas by passing it rapidly through bacterial emulsions for periods of 60 minutes, and found that no degree of germicidal action was manifested.

The results of a few experiments in which the discharge was sprayed on to the bacterial emulsion through an atmosphere of carbon dioxide are given in Table VIII.

Table VIII.

Species.	Current at 100 volts supplied to motor driving the alternator.	Duration of exposure to the discharge.	Result.
	ampères.	mins.	
B. typhosus	5	15	+
	6	30	0
	5	45	Scanty growth.
	5	60	0
	5	60	0
B. pyocyaneus	—	60	0
		60	0
B. prodigiosus	—	15	+
		30	+
		60	0
		60	0

When the discharge is sprayed on to the bacterial emulsion through an atmosphere of carbon dioxide, distinct germicidal effects are obtained in some experiments; but, as was the case when the discharge occurred in an atmosphere of hydrogen, we found that peroxide of hydrogen was taken up in solution in considerable quantities after the exposure of the emulsion for as short a period as 15 minutes. It is probable that under the action of the discharge carbon dioxide is decomposed into carbon monoxide, oxygen,* and possibly other products, and the hydrogen peroxide is then formed by the action of the oxygen in nascent condition on the water vapour present. Some other substance, which rapidly liberated iodine from iodide of potassium, was also present in the solution, but starch iodide papers suspended in the tubes above the emulsion gave no indication of the presence of ozone.

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* 'Journal of Pathology and Bacteriology,' vol. 1, p. 468 1893.

It is perhaps worth mentioning that the colour of the discharge spark in an atmosphere of carbon dioxide alters in appearance after the experiment has been in progress for a few minutes. At first the spark presents a peculiar green colour, but after a few minutes the appearance alters to that of a combination of green with a very pale violet colour, so that often whilst the upper part of the spark is of a distinct green colour the lower part appears to spread out in the form of a fine network of violet rays.

Experiments in an Atmosphere of Carbon Monoxide.

One series of experiments in which the discharge from the alternator was sprayed through an atmosphere of carbon monoxide on to emulsions containing *B. pyocyaneus* and *B. prodigiosus* respectively was carried out.

The carbon monoxide was prepared by acting on sodium formate with concentrated sulphuric acid, and the gas was passed slowly from the gasholder to another through tubes of the emulsions as they were being subjected to the action of the discharge. In every experiment of this series the non-sporing bacteria were destroyed after an exposure to the discharge for 30 minutes, the current supplied to the motor driving the alternator being 5 ampères, and the potential difference being 100 volts. In all these experiments, again, peroxide of hydrogen was found to be present in some quantity after the emulsion had been exposed to the action of the discharge for as short a period as 10 minutes. According to Berthelot,† carbon dioxide and other products are formed when a silent electrical discharge is passed through carbon monoxide.

Experiments in an Atmosphere of Nitrogen.

Two series of experiments in which *B. pyocyaneus* and *B. prodigiosus* were exposed to the action of the discharge in an atmosphere of nitrogen were carried out. In one series of experiments the gas, probably mixed with argon, etc., was obtained from common air by passing it over heated copper; in the other series the gas was prepared by heating a mixture of strong solutions of potassium nitrite and ammonium chloride, the gas being subsequently purified by passage through strong sulphuric acid. And in every experiment carried out under these conditions the bacteria were destroyed after exposure to the discharge for 30 minutes, the current being of amplitude similar to that used for the experiments in carbon monoxide.

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* Compare results obtained by Collie on sparking carbon dioxide in vacuum tubes ('Chem. Soc. Trans.,' 1901, p. 1063). Also compare Berthelot ('Compt. Rend.,' 1898, vol. 126).

† *Loc. cit.*

After exposure to the discharge the emulsions were found to be strongly acid in reaction and to contain quantities of nitrous and nitric acids which had probably been formed by the action of the discharge on nitrogen in the presence of water vapour.

IX.—Conclusions.

The results of our experiments may be summarised as follows:—

1. When bacteria suspended in water are exposed in an atmosphere of common air to the action of electrical discharges of high potential and rapid frequency, such as are used in medicine for purposes of treatment, sufficient quantities of nitrous and nitric acids are taken up in solution within as short a period as 15 minutes to sterilise the emulsion, and the germicidal action of these compounds in their nascent condition is favoured under ordinary circumstances (*a*) by the heating of the medium in which they are suspended by heat rays resulting from the discharge, and (*b*) by the concomitant formation of substances such as ozone and peroxide of hydrogen, which, readily yielding up a portion of their oxygen, accelerate the interaction of nitrous and nitric acids and their consequent germicidal activity.

2. When the bacteria are exposed to the action of the discharge in an atmosphere of pure hydrogen, under similar conditions, there is a decomposition of the water vapour necessarily present in the atmosphere of the tube with the formation of peroxide of hydrogen in quantities such as are sufficient after a time to exercise distinct germicidal action in the case of some bacteria.

3. When exposure to the discharge occurs in atmospheres of pure carbon dioxide or carbon monoxide there may again be sufficient peroxide of hydrogen formed to exercise germicidal action.

4. When exposure to the discharge occurs in an atmosphere of pure nitrogen, sterilisation may be effected by the action of nitrous and nitric acids.

5. The action on bacteria of the light rays resulting from the discharge is negligible under the time conditions of our experiments.

6. In all cases in which germicidal action was manifest it appeared to us that the result was due to the action of chemical substances formed by the discharge, either from the surrounding atmosphere or from the water in which the bacteria were suspended; and in no case did we obtain any evidence that, under the time conditions, the electrical current or its discharge had any direct injurious influence on the bacteria, apart from the accompanying formation of chemical germicidal substances and from whatever effect may be exercised by the heat rays.

In considering the application of the results of our experiments to the explanation of the results which are obtained in medical practice by the use of high-frequency discharges in the treatment of lupus and other diseases in which surface ulceration has occurred, the different conditions under which bacteria are exposed to the action of the discharge must be taken into account.

In our experiments the bacteria were immersed in a column of water of about 3 cm. depth, but the water would probably be in a state of continuous molecular disturbance, which would tend to bring the bacteria into intimate contact with the chemical substances which are being taken up in solution during the exposure.

In cases of lupus and the various conditions of ulceration in which the high-frequency discharges are used in medical practice the bacteria are either exposed in a film of albuminous fluid on the surface under treatment, or may be more or less deeply embedded in granulation tissue. When bacteria are exposed on the surface it is obvious that the germicidal action of nitrous and nitric acids formed in the air as the result of the discharge will be readily and effectually exercised; and even when the bacteria are embedded in granulation tissue at a slight depth from the surface we think that it is possible that some penetration of the tissue by these substances in a nascent and active condition may occur, favoured doubtless by the "impact" action of the discharge. And, in any case, we feel justified in assuming as the result of our experiments that under the time conditions which prevail in the use of these discharges in medical practice the electric current itself has no special or direct injurious influence on the bacteria present, and that, therapeutically, treatment by the use of these discharges under the given conditions must be regarded merely as an efficient means for the intimate application of chemical germicides formed from the atmosphere in which the discharge takes place. Under the conditions existing in practice the heat rays resulting from the discharge also probably assist in the germicidal action in some degree.

The use of these discharges in conditions of disease, such as carcinoma and sarcoma, not due to bacterial infections, has not come within the scope of our present inquiry, but we may say that we think it probable that when the discharge is sprayed on to the unbroken skin there may be same local absorption of nitrous compounds under the influence of the discharge, and that these may have some effect upon cells immediately underlying the area of skin to which the discharges are applied. The "skinning over" of the ulcerated surface of malignant new growths, without much, or any, effect upon the underlying new formation, which has occasionally been observed, may be attributed to the chemical germicidal action affecting the bacteria to which the ulceration is due.

We have to express our thanks to Dr. C. R. C. Lyster, the Medical Officer in charge of the Electrical Department at the Middlesex Hospital, for his kindness in placing the electrical apparatus at our disposal for these experiments.

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An Investigation into the Structure of the Lumbo-sacral-coccygeal Cord of the Macaque Monkey (Macacus sinicus).

By Mabel Purefoy FitzGerald.

(Communicated by Professor Francis Gotch, M.A., D.Sc., F.R.S. Received June 17,—Read December 14, 1905.)

The following measurements of the cross-sections of the lumbo-sacral-coccygeal cord of the Macaque monkey (*Macacus sinicus*), with the calculations made therefrom, are here given as the preliminary results of a research made upon the spinal cord of this monkey.

For the purpose of presenting the facts as clearly as possible, the paper is illustrated by a series of curves based upon the above-mentioned records.

The method of using curves to represent the areas of the grey and white substance at different levels of the cord has been adopted by former investigators, namely, by Woroschiloff,* Lüderitz,† Krause and Aguerre,‡ and more recently by Donaldson and Davis§ in their paper published whilst this work was in the process of completion. The above-mentioned curves were constructed from measurements of the human spinal cord; the curves of Woroschiloff, Donaldson and Davis, and in part those of Lüderitz, being based upon the measurements published by Stilling|| in his classical work on the structure of the spinal cord.

It was intended to include a comparison of the human spinal cord with that of the monkey in this paper, but it is now thought advisable to publish the results of these investigations separately. The work done in this direction was also based upon Stilling's¶ measurements of human spinal cords.

Before entering into the details of the monkey's spinal cord, the methods employed in the research are here described as briefly as possible.

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* Woroschiloff, "Der Verlauf der Motorischen und Sensiblen Bahnen durch das Lendenmark des Kaninchens," 'Berichte über die Verhandlungen der Königlich Sächsischen Gesellsch. der Wissenschaften zu Leipzig,' vol. 26, 1874.

† Lüderitz, C., "Über das Rückenmarksegment. Ein Beitrag zur Morphologie und Histologie des Rückenmarks," 'Archiv f. Anatomie u. Entwickelungsgeschichte,' Anat. Abtheil., 1881, pp. 423—495.

‡ Krause, R., and Aguerre, J., "Untersuchungen über den Bau des menschlichen Rückenmarkes mit besonderer Berücksichtigung der Neuroglia," 'Anatomischer Anzeiger,' vol. 18, 1900.

§ Donaldson, H. H., and Davis, D. J., "A Description of Charts showing the Areas of the Cross-sections of the Human Spinal Cord at the Level of each Spinal Nerve," 'The Journal of Comparative Neurology,' vol. 13, No. 1, 1903.

|| Stilling, B., 'Neue Untersuchungen über den Bau des Rückenmarkes,' Cassel, 1859.

¶ Stilling, B., *ibid.*

Technique.—The monkey was injected by Mann's* method, with picro-corrosive formaldehyde solution:—†

Formula.

Boiling water	100 c.c.
Sublimate	2·5 grammes.

When dissolved, add—

Picric acid	1 gramme.

Allow to cool, and immediately before use add—

Formol	10 c.c.

The spinal cord was allowed to remain in the vertebral canal until the following day, when it was removed with the spinal ganglia, and placed in 50 per cent. alcohol. The cord was then cut at different levels, and the various portions of it were dehydrated through gradual transference to alcohols of increasing strength, cleared in xylol, and embedded in paraffin (melting point, 52°). The cord was found to be well fixed throughout its length.

The lumbo-sacral-coccygeal cord was cut in serial sections, 10 μ and 20 μ thick, with the large Cambridge rocking microtome, which cuts flat sections, and the sections mounted on albuminised slides.‡ A certain number of serial sections were also cut at each of the levels of the dorsal (thoracic) and cervical cord, and similarly mounted.

After the removal of the paraffin by xylol, the sections were treated with absolute alcohol and then with iodine potassium iodide solution to remove the excess of corrosive sublimate and to decompose the albuminates of mercury. The iodine solution was removed with methylated spirit and distilled water. The sections were stained with eosin and toluidin blue§ (eosin, 10 minutes; toluidin blue, 2½ minutes; or for a longer time in similar proportions), and mounted in xylol balsam.

Three drawings were made at the level of each pair of spinal nerves, the sections being selected from the upper, middle, and lower portion of the line of entrance of the dorsal nerve root. The drawings were made with an Abbé Camera Lucida (Zeiss), a Zeiss microscope with the tube at 158, a No. 4 compensating ocular, and a Wray 3-inch lens. The magnification was equal to 20 diameters, except in dealing with certain sections in the lower regions of the cord, when it became advisable to use a magnification equal to 40 diameters. For this purpose a Leitz No. 1 lens was employed. The drawing paper was placed on a Bernhard drawing board (Zeiss), the board being level.

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[*] Mann, G., 'Zeit. f. Wiss. Mikr.,' vol. 11, p. 482 (1894).

[†] Mann, G., 'Methods and Theory of Physiological Histology,' 1902, p. 97.

[‡] Mann, G., 'Zeit. f. Wiss. Mikr.,' vol. 11, 1895, pp. 479—494.

[§] Mann, G., *ibid.*

For taking the measurements of the drawings Amsler's planimeter No. 3 was used, so adjusted that one division on the disc indicated 100 sq. cm., and in those cases where the areas to be measured were very small the planimeter was set so that one division on the disc indicated 20 sq. cm. Every care was taken to obtain accuracy. In the majority of cases the pointer was carried five times round the area to be measured, and ten or more times when the areas were small. To guard against accidental errors the planimeter reading was always taken after completing the first circuit, and the result compared with that obtained by dividing the final reading by the number of times the pointer had been carried round. The difference was in all cases within the limits of instrumental error.

In each case the total area of the cross-sections of the cord was first determined. The sectional areas of the dorsal columns, the ventro-lateral columns, and the grey substance were then each measured separately in each half of the cord, the sum of the two halves being taken as the measurement of the whole.

The total area of the central canal was measured independently of the grey substance, and the results are given in Tables II, VI. These measurements are not separately represented in the charts, though it is necessary to take them into account in explaining the form of some of the curves.

The difference between the sum total of the measurements of the component parts of the cord and of the total section area was, in each case, noted as "error." As a rule the error was negative, its average value being 0·2 per cent. This may be regarded as due to "personal equation."

The total sectional area of the white substance was obtained by adding together the figures obtained for the dorsal and the ventro-lateral columns.

It must be here stated that the term "ventro-lateral" columns is used in this paper to signify the whole of the white substance ventral to Lissauer's "marginal zone." Lissauer* regarded this zone (Waldeyer's "Markbrücke") as belonging to the dorsal horn and forming a bridge between the dorsal and lateral strands of the white substance. It has therefore been used as a boundary between the two portions of the white substance, and has been measured with, and as a part of, the grey substance.

For measuring the grey substance in each half of the cord, the division was made by continuing a line from the dorsal median septum to the dorsal boundary of the central canal, and from the ventral boundary of this to the ventral median fissure. The grey substance was subsequently subdivided for the purpose of obtaining information as to the relative development of the "dorsal" and of the "ventral" horns. Since no actual boundary exists between the dorsal and the ventral portions of the grey substance, an arbitrary division was made as follows: In each half of the cord a line was drawn from the middle of the central canal to that point where the white substance projects furthest into the grey substance (see fig. 1). The grey substance dorsal to this was designated "dorsal horns," and that ventral to it the "ventral horns." From the seventh lumbar region (middle portion) downwards, a modification of this method was rendered necessary by the presence of the fibres of the formatio reticularis. The lines were therefore drawn from the central canal, in the manner already described, to the corresponding points which were still discernible, and then continued in a horizontal direction through the fibres of the formatio reticularis (see fig. 2).

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* Lissauer, 'Archiv f. Psychiatrie u. Nervenkrankheiten,' vol. 17, 1886.

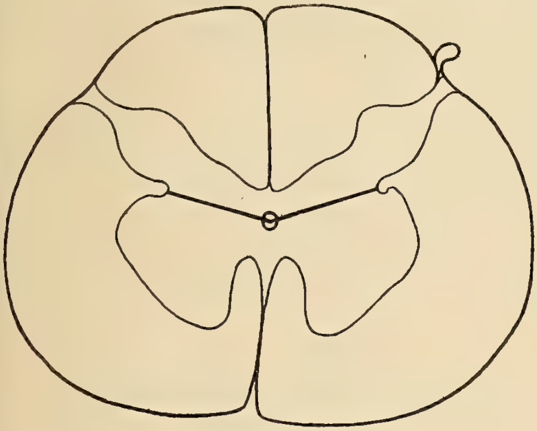

FIG. 1.

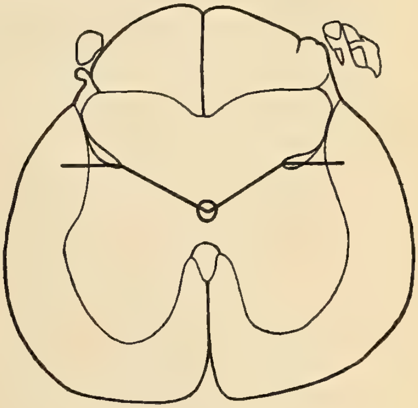

FIG. 2.

The "ventral horns" were measured in each case and the section area of the "dorsal horns" determined by subtracting the figures obtained for the "ventral horns" from the sum of the total grey substance. The "dorsal horns" therefore include Lissauer's "marginal zone," the substantia spongiosa and the substantia gelatinosa Rolandi.

Owing to the lack of sufficient differentiation, it was not possible to make an additional measurement of the substantia gelatinosa Rolandi. In a "control" monkey, fixed with picro-corrosive formaldehyde solution containing 20 c.c. instead of 10 c.c. formol for 100 c.c. of picro-corrosive solution, it appears to be possible to do this, but the work has not been carried out as yet.

Each table of figures is accompanied by a chart with curves, representing the same graphically.

In drawing the curves the same type of line and the same lettering have, as far as possible, been used throughout the series to denote the same constituent of the cord. The curves are to be read from left to right.

The number of nerve roots in the spinal cord of the Macaque monkey is as follows: 7 cervical, 12 dorsal, 7 lumbar, 4 sacral and 3 coccygeal.

The portion of the cord from which each pair of nerves arise has been spoken of throughout this paper as the corresponding "region."

The particular portion of the cord examined extended from the upper limit of the region of the first lumbar nerve to the lower limit of the third coccygeal region and measured approximately 6·3 cm.

In the charts dealing with section areas and with percentages (Charts I to IV and XIII, XIV), the length of the cord is expressed along the abscissa line in centimetres and parts of a centimetre. The uppermost section of the first lumbar region is taken as zero. The distance from zero to each measured section is given in centimetres or parts of a centimetre in a separate column in each table of figures (except in those where only mean results are quoted), and on the curves the observation points are marked by a dot or cross as nearly as possible in the exact position indicated in the table.

As a matter of convenience, the first drawing made in the first lumbar region was of a section occurring at 0·142 cm. below that taken as zero.

In the section area and percentage charts which give the complete results obtained (Charts I to IV and XIII, XIV), the numbers I, II, III, IV, etc., mark on the zero abscissa line the approximate cephalic end of the region of each pair of spinal nerves.

In the charts where the curves are constructed from mean figures (Charts V to XII, and XV to XX), the length of the cord and of the individual nerve regions has been disregarded, and the calculations referring to the region of each nerve (marked I, II, III, etc.) are plotted at equal distances along the abscissa.

The paper is arranged in the following order:—

A 1. Areas of the cross-sections of the spinal cord and of the grey and of the white substance in the region of each spinal nerve.
  2. Percentage of the grey and of the white substance in the cross-section of the cord.
  3. Ratio of the total white substance, and of the dorsal and of the ventro-lateral white columns, to the grey substance.
  4. Ratio of the dorsal and of the ventro-lateral white columns to the total white substance.
  5. Ratio of the ventro-lateral to the dorsal columns.

B 1. Section areas of the dorsal and of the ventral horns of the grey substance.
  2. Percentage of the dorsal and of the ventral horns in the total grey substance.

3. Ratio of the ventral to the dorsal horns.
4. Ratio of the dorsal and of the ventral horns to the total area of the cross-section of the cord.
5. Ratio of the dorsal and of the ventro-lateral columns to the dorsal horns.
6. Ratio of the dorsal and of the ventro-lateral columns to the ventral horns.
7. Relative increase and decrease in the sectional area of the cord and of each of its component parts.

C 1. Diagrams. General configuration of the lumbo-sacral and coccygeal cord in sectional area.

2. Outline drawings of the cross-sections of the cord in the region of each spinal nerve.

A1.—*Areas of the Cross-sections of the Spinal Cord and of the Grey and of the White Substance in the Region of each Spinal Nerve.*

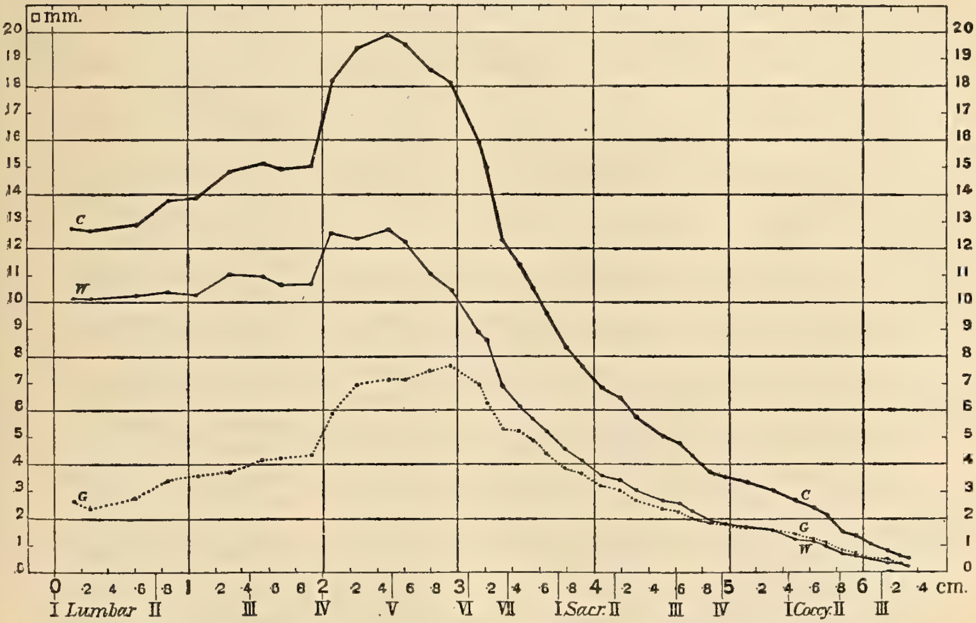

CHART I.—Curves showing the Areas of the Cross-sections of the Spinal Cord and of the Grey and of the White Substance (Tables I, II, III).

The ordinates denote section area in square millimetres, the abscissæ the length of the cord in centimetres measured from the uppermost section in the first lumbar region. The numbers (I, II, III, etc.) mark on abscissa line the approximate cephalic end of the region of each pair of spinal nerves.

Total area of the cross-section of the cord......	C, thick continuous line.
Section area of the grey substance	G, dotted line.
Section area of the white substance	W, thin continuous line.

(a) *Areas of the Cross-sections of the Spinal Cord.* (C.)

Table I.—Areas of the Cross-sections of the Cord (Charts I and II).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	39·04	9·76	
XII	—	45·82	11·46	
Lumbar—				
I Upper	0·142	50·94	12·74	12·76
Middle	0·268	50·64	12·66	
Lower	0·608	51·58	12·90	
II Upper	0·846	55·22	13·81	14·17
Middle	1·058	55·46	13·87	
Lower	1·302	59·40	14·85	
III Upper	1·558	60·42	15·11	15·03
Middle	1·681	59·66	14·92	
Lower	1·918	60·30	15·08	
IV Upper	2·064	72·86	18·22	19·16
Middle	2·244	77·58	19·40	
Lower	2·484	79·50	19·88	
V Upper	2·608	78·00	19·50	18·75
Middle	2·794	74·40	18·60	
Lower	2·944	72·60	18·15	
VI Upper	3·154	63·64	15·91	14·42
Middle	3·205	60·06	15·02	
Lower	3·328	49·32	12·33	
VII Upper	3·450	45·66	11·42	10·54
Middle	3·557	42·32	10·58	
Lower	3·642	38·48	9·620	
Sacral—				
I Upper	3·782	33·50	8·375	7·693
Middle	3·906	31·44	7·860	
Lower	4·057	27·38	6·845	
II Upper	4·197	25·86	6·465	5·758
Middle	4·316	23·14	6·785	
Lower	4·509	20·10	5·025	
III Upper	4·623	19·20	4·800	4·310
Middle	4·723	17·44	4·360	
Lower	4·858	15·08	3·770	
IV Upper	4·974	14·20	3·550	3·325
Middle	5·138	13·40	3·350	
Lower	5·312	12·30	3·075	
Coccygeal—				
I Upper	5·486	10·76	2·690	2·403
Middle	5·622	9·56	2·390	
Lower	5·726	8·52	2·130	
II Upper	5·847	24·08	1·505	1·319
Middle	5·939	22·18	1·386	
Lower	6·058	17·04	1·065	
III Upper	6·174	3·34	0·8350	0·6679
Middle	6·278	10·34	0·6462	
Lower	6·331	2·09	0·5225	

The section area of the spinal cord (C) (Charts I, II, Table I) increases from the region of the first lumbar to the lower portion of that of the fourth lumbar nerve, where the maximum size of the lumbar enlargement is reached (19·88 sq. mm.).

By comparing the measurements of the first lumbar region with the measurements of the twelfth dorsal (thoracic) given in the accompanying table, it will be seen, that the increase in the section area of the cord has begun prior to the region of the first lumbar nerve.

The increase is gradual to the lower portion of the third lumbar region. It becomes very marked in the fourth, for when the maximum is reached the section area of the cord is more than one and a-half times as great as in the first lumbar region. The section area remains very much the same (19·5 sq. mm.) from the middle portion of the fourth to the upper portion of the fifth lumbar region. The gradual decrease in section area in the remaining portions of the fifth is followed by a marked diminution in the sixth lumbar region. The section area in the lower portion of the sixth (12·33 sq. mm.) is rather less than that in the first lumbar region. Continuing with rapidity through the regions of the seventh lumbar and the first sacral nerves, the decrease becomes more and more gradual as the lower regions of the cord are reached. The section area in the lower portion of the third coccygeal region is about 1/40th that in the fourth lumbar region (*i.e.*, 0·5—19·88 sq. mm.).

The section area of the grey substance (G) (Charts I, II, Table II) increases from the region of the first to that of the fifth lumbar nerve, and is the potent factor in the enlargement of the total section area of the cord.

The maximum (7·65 sq. mm.) is reached in the lower portion of the fifth lumbar region and it is noteworthy that this is found at a lower level than the maximum section areas of the cord and of the white substance.

The grey substance decreases in section area from the fifth lumbar region to the end of the cord.

The increase in section area, though continuous, is gradual from the first to the third lumbar region (lower part), becomes rapid in the fourth, and is again gradual in the fifth lumbar region. In the middle portion of the fourth and the upper portion of the sixth lumbar region the section area is the same (*ca.* 7 sq. mm.).

The decrease is rapid from the fifth to the lower portion of the region of the sixth lumbar nerve, after which the reduction of section area takes place very gradually.

In the fifth lumbar region (maximum) the section area is about three times as great as that in the first lumbar region (upper part) (*ca.* 7·6 to 2·6 sq. mm.); in the second sacral it is much the same as in the first lumbar, and in the third coccygeal region is about 1/30th that in the fifth lumbar.

(b) *Section Area of the Grey Substance.* (G.)

Table II.—Section Area of the Grey Substance and of the Central Canal (Charts I, II, V, XIII).

Region of cord.	Distance in cm. from the uppermost section of I Lumbar region.	Grey substance.		Mean.	Central canal.	
		Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.		Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.
Dorsal (thoracic)—						
VI	—	4 ·98	1 ·245	—	0 ·02	0 ·0050
XII	—	7 ·56	1 ·890	—	0 ·04	0 ·0100
Lumbar—						
I Upper ...	0 ·142	10 ·22	2 ·555		0 ·08	0 ·0200
I Middle ...	0 ·268	9 ·94	2 ·485	2 ·607	0 ·06	0 ·0150
I Lower ...	0 ·608	11 ·12	2 ·780		0 ·06	0 ·0150
II Upper	0 ·846	13 ·58	3 ·395		0 ·03	0 ·0075
II Middle ...	1 ·058	14 ·20	3 ·550	3 ·553	0 ·04	0 ·0100
II Lower ...	1 ·302	14 ·86	3 ·715		0 ·11	0 ·0275
III Upper ...	1 ·558	16 ·64	4 ·160		0 ·14	0 ·0350
III Middle ...	1 ·681	16 ·90	4 ·225	4 ·240	0 ·08	0 ·0200
III Lower ...	1 ·918	17 ·34	4 ·335		0 ·08	0 ·0200
IV Upper ...	2 ·064	23 ·42	5 ·855		0 ·08	0 ·0200
IV Middle ...	2 ·244	27 ·86	6 ·965	6 ·651	0 ·08	0 ·0200
IV Lower ...	2 ·484	28 ·53	7 ·133		0 ·16	0 ·0400
V Upper ...	2 ·608	28 ·60	7 ·150		0 ·14	0 ·0350
V Middle ...	2 ·794	29 ·74	7 ·435	7 ·410	0 ·18	0 ·0450
V Lower ...	2 ·944	30 ·58	7 ·645		0 ·18	0 ·0450
VI Upper ...	3 ·154	27 ·90	6 ·975		0 ·12	0 ·0300
VI Middle ...	3 ·205	25 ·49	6 ·373	6 ·234	0 ·12	0 ·0200
VI Lower ...	3 ·328	21 ·42	5 ·355		0 ·14	0 ·0350
VII Upper ...	3 ·450	20 ·84	5 ·210		0 ·08	0 ·0200
VII Middle ...	3 ·557	19 ·45	4 ·863	4 ·809	0 ·09	0 ·0225
VII Lower ...	3 ·642	17 ·42	4 ·355		0 ·12	0 ·0300
Sacral—						
I Upper ...	3 ·782	15 ·28	3 ·820		0 ·08	0 ·0200
I Middle ...	3 ·906	14 ·74	3 ·685	3 ·573	0 ·04	0 ·0100
I Lower ...	4 ·057	12 ·86	3 ·215		0 ·04	0 ·0100
II Upper ...	4 ·197	12 ·14	3 ·035		0 ·03	0 ·0075
II Middle ...	4 ·316	10 ·74	2 ·685	2 ·697	0 ·03	0 ·0075
II Lower ...	4 ·509	9 ·48	2 ·370		0 ·03	0 ·0075
III Upper ...	4 ·623	8 ·90	2 ·225		0 ·04	0 ·0100
III Middle ...	4 ·723	8 ·34	2 ·085	2 ·050	0 ·02	0 ·0050
III Lower ...	4 ·858	7 ·36	1 ·840		0 ·04	0 ·0100
IV Upper ...	4 ·974	6 ·98	1 ·745		0 ·03	0 ·0075
IV Middle ...	5 ·138	6 ·66	1 ·665	1 ·642	0 ·03	0 ·0075
IV Lower ...	5 ·312	6 ·06	1 ·515		0 ·03	0 ·0075
Coccygeal—						
I Upper ...	5 ·486	5 ·66	1 ·415		0 ·03	0 ·0075
I Middle ...	5 ·622	5 ·06	1 ·265	1 ·267	0 ·03	0 ·0075
I Lower ...	5 ·726	4 ·48	1 ·120		0 ·03	0 ·0075
II Upper ...	5 ·847	12 ·34	0 ·7712		0 ·08	0 ·0050
II Middle ...	5 ·939	11 ·55	0 ·7218	0 ·6797	0 ·08	0 ·0050
II Lower ...	6 ·058	8 ·74	0 ·5462		0 ·10	0 ·0062
III Upper ...	6 ·174	1 ·70	0 ·4250		0 ·03	0 ·0075
III Middle ...	6 ·278	4 ·92	0 ·3075	0 ·3266	0 ·07	0 ·0043
III Lower ...	6 ·331	0 ·99	0 ·2475		0 ·02	0 ·0050

(c) *Section Area of the White Substance.* (W.)

Table III.—Section Area of the White Substance (Charts I and VI).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	33·88	8·470	
XII	—	38·24	9·560	
Lumbar—				
I Upper	0·142	40·52	10·13	10·12
Middle	0·268	40·64	10·16	
Lower	0·608	40·26	10·07	
II Upper	0·846	41·54	10·39	10·58
Middle	1·058	41·18	10·30	
Lower	1·302	44·26	11·07	
III Upper	1·558	43·62	10·91	10·76
Middle	1·681	42·68	10·67	
Lower	1·918	42·80	10·70	
IV Upper	2·064	49·30	12·33	12·46
Middle	2·244	49·54	12·39	
Lower	2·484	50·70	12·68	
V Upper	2·608	49·06	12·27	11·27
Middle	2·794	44·32	11·08	
Lower	2·944	41·90	10·48	
VI Upper	3·154	35·62	8·905	8·153
Middle	3·205	34·49	8·623	
Lower	3·328	27·72	6·930	
VII Upper	3·450	24·60	6·150	5·682
Middle	3·557	22·68	5·670	
Lower	3·642	20·90	5·225	
Sacral—				
I Upper	3·782	18·12	4·530	4·097
Middle	3·906	16·66	4·165	
Lower	4·057	14·38	3·595	
II Upper	4·197	13·64	3·410	3·047
Middle	4·316	12·32	3·080	
Lower	4·509	10·60	2·650	
III Upper	4·623	10·24	2·560	2·250
Middle	4·723	9·10	2·275	
Lower	4·858	7·66	2·915	
IV Upper	4·974	7·14	1·785	1·667
Middle	5·138	6·68	1·670	
Lower	5·312	6·18	1·545	
Coccygeal—				
I Upper	5·486	5·02	1·255	1·123
Middle	5·622	4·48	1·120	
Lower	5·726	3·98	0·9950	
II Upper	5·847	11·72	0·7325	0·6345
Middle	5·939	10·58	0·6612	
Lower	6·058	8·16	0·5099	
III Upper	6·174	1·59	0·3975	0·3345
Middle	6·278	5·38	0·3362	
Lower	6·331	1·08	0·2700	

The section area of the white substance (W) (Chart I, Table III) increases from the region of the first lumbar to that of the fourth lumbar nerve, and decreases from the latter region to the end of the cord. The increase in the white substance (W) is not so great as that of the grey substance (G).

There is very little alteration in the section area of the white substance through the first and the greater part of the second lumbar regions, but a marked increase takes place between the lower portion of the third and the fourth and in the fourth lumbar region. The chief development of the white substance extends through the fourth and the upper portion of the fifth lumbar region. The maximum (12·68 sq. mm.) being reached in the lower portion of the fourth, is thus found in the same region as the maximum section area of the cord.

The decrease is rapid in the greater part of the fifth lumbar, the section area of the white substance in the lower part of this region (10·48 sq. mm.) being about the same as that in the first lumbar.

Attention must be drawn to the fact that whilst this marked decrease is taking place in the section area of both the white substance and of the cord in the fifth lumbar region, the section area of the grey substance is still increasing.

The rapid diminution of the section area of the white substance continues to the seventh lumbar region; after this the decrease is gradual.

From the seventh lumbar to the third sacral region (lower) the section area of the white substance (W) is only slightly greater than that of the grey substance (G), whilst from the fourth sacral region to the end of the cord the section areas of the two substances are almost the same.

In Chart I, and in the above description, the white substance (W) has been regarded as a whole. Through the remainder of the paper it is also dealt with in two distinct parts—*i.e.*, dorsal columns and ventro-lateral columns. The latter term is here used, as before stated (p. 90), to signify the whole of the white substance other than the dorsal columns. By this means it is possible to obtain information as to the relative value of the contributions made to the white substance of the cord by dorsal roots and by central grey substances respectively.*

The section areas of the dorsal columns and of the ventro-lateral columns are shown in the following chart (II):—

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* It must be remembered that, owing to the presence of some endogenous fibres amongst those of extra-spinal origin in the dorsal columns, the comparison can only be regarded as of general value.

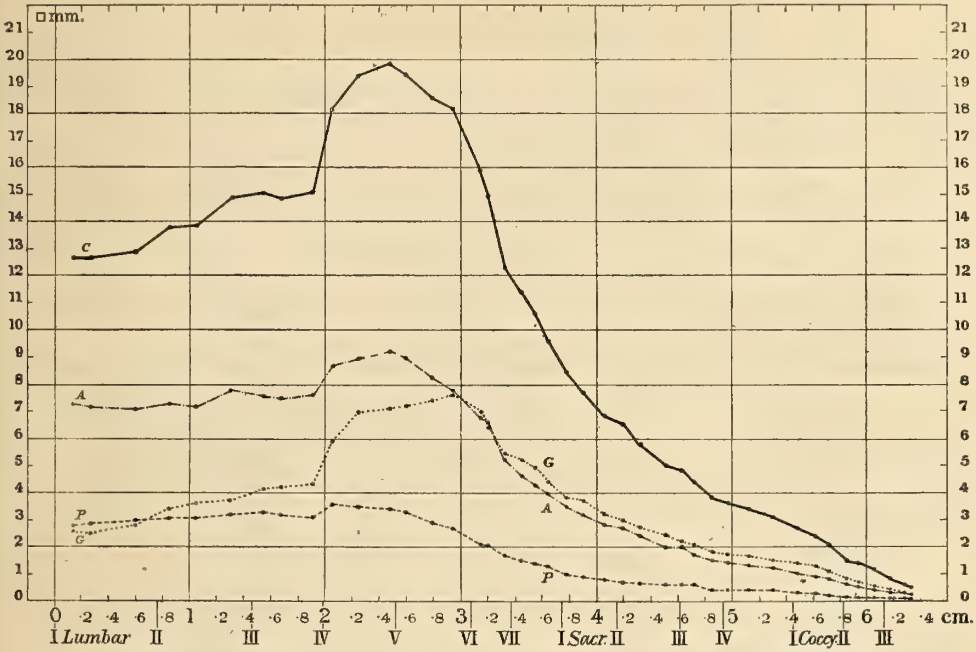

CHART II.—Curves showing the Areas of the Cross-sections of the Cord, of the Grey Substance, and of the Dorsal and the Ventro-lateral Columns of the White Substance (Tables I, II, IV, V).

The ordinates denote section area in square millimetres, the abscissæ the length of the cord in centimetres measured from the uppermost section in the first lumbar region. The numbers (I, II, III, etc.) mark approximately the cephalic end of the region of each pair of spinal nerves.

Total area of the cross-section of the cord	C, thick continuous line.
Section area of the grey substance	G, dotted line.
Section area of the dorsal white columns...............	P, broken line.
Section area of the ventro-lateral white columns ...	A, line and dot.

The section area of the dorsal columns (P) (Chart II, Table IV), is 2·81 sq. mm. in the upper portion of the first lumbar region, and increases gradually from this to the fourth lumbar, where it reaches its maximum, 3·6 sq. mm.

The maximum thus occurs in the region of the same lumbar nerve as the maximum section areas of the cord and of the white substance; but it differs in position to these, being found in the upper instead of the lower portion of the fourth lumbar region. It occurs at some distance, therefore, from the maximum section area of the grey substance (*v.* L., lower).

The larger areas of the dorsal columns extend through the fourth and the upper portion of the fifth lumbar region. The section area in the middle portion of the latter region is much the same as that in the first lumbar.

(d) *Section Area of the Dorsal Columns.* (P.)

Table IV.—Section Area of the Dorsal Columns (Charts II and VII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	8·84	2·210	
XII	—	10·46	2·615	
Lumbar—				
I Upper	0·142	11·24	2·810	2·895
Middle	0·268	11·68	2·920	
Lower	0·608	11·82	2·955	
II Upper	0·846	12·52	3·130	3·138
Middle	1·058	12·22	3·055	
Lower	1·302	12·92	3·230	
III Upper	1·558	13·18	3·295	3·195
Middle	1·681	12·72	3·180	
Lower	1·918	12·44	3·110	
IV Upper	2·064	14·44	3·610	3·508
Middle	2·244	13·94	3·485	
Lower	2·484	13·72	3·430	
V Upper	2·608	13·06	3·265	2·952
Middle	2·794	11·46	2·865	
Lower	2·944	10·90	2·725	
VI Upper	3·154	8·56	2·140	1·960
Middle	3·205	8·28	2·070	
Lower	3·328	6·68	1·670	
VII Upper	3·450	6·06	1·515	1·385
Middle	3·557	5·54	1·385	
Lower	3·642	5·02	1·255	
Sacral—				
I Upper	3·782	4·08	1·020	0·9016
Middle	3·906	3·68	0·9200	
Lower	4·057	3·06	0·7650	
II Upper	4·197	2·92	0·7300	0·6783
Middle	4·316	2·78	0·6950	
Lower	4·509	2·44	0·6100	
III Upper	4·623	2·24	0·5600	0·5183
Middle	4·723	2·20	0·5500	
Lower	4·858	1·78	0·4450	
IV Upper	4·974	1·64	0·4100	0·3866
Middle	5·138	1·54	0·3850	
Lower	5·312	1·46	0·3650	
Coccygeal—				
I Upper	5·486	1·08	0·2700	0·2433
Middle	5·622	1·02	0·2550	
Lower	5·726	0·82	0·2050	
II Upper	5·847	2·36	0·1475	0·1291
Middle	5·939	2·22	0·1387	
Lower	6·058	1·62	0·1012	
III Upper	6·174	0·27	0·0675	0·0529
Middle	6·278	0·78	0·0487	
Lower	6·331	0·17	0·0425	

The decrease in the section area of the dorsal columns is much more rapid than the increase, and, beginning in the fourth lumbar region, is continuous to the end of the cord. The decrease is most marked between the fifth lumbar and the first sacral regions, and becomes very gradual in the lower sacral and in the coccygeal regions.

In the first lumbar region the section area of the dorsal columns (P) is greater than that of the grey substance (G). This is also the case in both the sixth and twelfth dorsal (thoracic) regions (see Tables II and IV). In the second lumbar region, and throughout the remainder of the cord below this, the section area of the dorsal columns is less than that of the grey substance.

It is of interest to note that the section area of the dorsal columns in the greater part of the sixth lumbar region is approximately the same as that in the sixth dorsal (thoracic).

The section area of the ventro-lateral columns (A) (Chart II, Table V) remains about the same through the first and the greater part of the second lumbar region (*ca.* 7·2 sq. mm.). It is somewhat larger in the third, and increases rapidly from the lower portion of this and throughout the fourth lumbar region.

The maximum (9·25 sq. mm.) is reached in the lower portion of the fourth lumbar, and thus occurs in the same region as the maximum section areas of the cord, white substance, and dorsal columns.

The chief development of the ventro-lateral columns extends from the third lumbar (lower) to the lower portion of the fifth lumbar region, the section area in the upper portion of the fifth being nearly the same as that in the lower portion of the fourth.

The section area diminishes from the fourth lumbar region (lower part) to the end of the cord. The decrease is rapid to the third sacral region, and is most marked in the fifth, sixth, and seventh lumbar regions.

From the first to the fifth lumbar region inclusive, the section area of the ventro-lateral columns (A) exceeds that of the grey substance (G), especially in the first three lumbar regions. The section areas are approximately equal in the sixth lumbar region. From the seventh lumbar region to the end of the cord the section area of the ventro-lateral columns is less than that of the grey substance, but the areas approach each other very nearly in the lower coccygeal regions.

The increase in the section area of the whole white substance (Chart I, W) between the first and the fourth lumbar, and the decrease between the fourth and the sixth lumbar regions, is chiefly due to the variation in the ventro-lateral columns (Chart II, A). The actual increase and decrease in the section area of these columns (A) being about twice as great as that of the dorsal columns (P).

(e) *Section Area of the Ventro-lateral Columns.* (A).

Table V.—Section Area of the Ventro-lateral Columns (Charts II and VIII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	25·04	6·260	
XII	—	27·78	6·945	
Lumbar—				
I Upper	0·142	29·28	7·320	7·223
Middle	0·268	28·96	7·240	
Lower	0·608	28·44	7·110	
II Upper	0·846	29·02	7·255	7·443
Middle	1·058	28·96	7·240	
Lower	1·302	31·34	7·835	
III Upper	1·558	30·44	7·610	7·563
Middle	1·681	29·96	7·490	
Lower	1·918	30·36	7·590	
IV Upper	2·064	34·86	8·715	8·953
Middle	2·244	35·60	8·900	
Lower	2·484	36·98	9·245	
V Upper	2·608	36·00	9·000	8·322
Middle	2·794	32·86	8·215	
Lower	2·944	31·00	7·750	
VI Upper	3·154	27·06	6·765	6·193
Middle	3·205	26·21	6·553	
Lower	3·328	21·04	5·260	
VII Upper	3·450	18·54	4·635	4·297
Middle	3·557	17·14	4·285	
Lower	3·642	15·88	3·970	
Sacral—				
I Upper	3·782	14·04	3·510	3·195
Middle	3·906	12·98	3·240	
Lower	4·057	11·32	2·840	
II Upper	4·197	10·72	2·680	2·368
Middle	4·316	9·54	2·385	
Lower	4·509	8·16	2·040	
III Upper	4·623	8·00	2·000	1·732
Middle	4·723	6·90	1·725	
Lower	4·858	5·88	1·470	
IV Upper	4·974	5·50	1·375	1·280
Middle	5·138	5·14	1·285	
Lower	5·312	4·72	1·180	
Coccygeal—				
I Upper	5·486	3·94	0·9850	0·8800
Middle	5·622	3·46	0·8650	
Lower	5·726	3·16	0·7900	
II Upper	5·847	9·36	0·5850	0·5051
Middle	5·939	8·36	0·5225	
Lower	6·058	6·54	0·4087	
III Upper	6·174	1·32	0·3300	0·2816
Middle	6·278	4·60	0·2875	
Lower	6·331	0·91	0·2275	

A2. The Percentage of the Grey and of the White Substance in the Cross-section of the Cord.

Table VI.—Percentage of the Grey and of the White Substances and of the Central Canal in the Cross-sections of the Cord. The total area of each cross-section of the cord = 100 (Charts III, IV, V, and VI).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Percentage of grey substance.	Mean percentage.	Percentage of white substance.	Mean percentage.	Percentage of the central canal.
Dorsal (thoracic)—						
VI	—	12·76	—	86·78	—	0·051
XII	—	16·50	—	83·46	—	0·087
Lumbar—						
I Upper ...	0·142	20·06	20·42	79·54	79·28	0·157
Middle ...	0·268	19·63		80·25		0·118
Lower ...	0·608	21·56		78·05		0·116
II Upper ...	0·846	24·59	25·07	75·23	74·66	0·054
Middle ...	1·058	25·60		74·25		0·072
Lower ...	1·302	25·02		74·51		0·185
III Upper ...	1·558	27·54	28·21	72·19	71·57	0·231
Middle ...	1·681	28·33		71·54		0·134
Lower ...	1·918	28·76		70·98		0·132
IV Upper ...	2·064	32·14	34·65	67·66	65·10	0·109
Middle ...	2·244	35·91		63·86		0·103
Lower ...	2·484	35·89		63·77		0·201
V Upper ...	2·608	36·67	39·59	62·90	60·06	0·179
Middle ...	2·794	39·97		59·57		0·241
Lower ...	2·944	42·12		57·71		0·247
VI Upper ...	3·154	43·84	43·25	55·97	56·53	0·188
Middle ...	3·205	42·48		57·41		0·199
Lower ...	3·328	43·43		56·20		0·283
VII Upper ...	3·450	45·64	45·62	53·88	53·93	0·175
Middle ...	3·557	45·96		53·59		0·212
Lower ...	3·642	45·27		54·31		0·311
Sacral—						
I Upper ...	3·782	45·61	46·49	54·09	53·20	0·238
Middle ...	3·906	46·88		52·99		0·127
Lower ...	4·057	46·97		52·52		0·146
II Upper ...	4·197	46·95	46·84	52·75	52·91	0·116
Middle ...	4·316	46·41		53·24		0·129
Lower ...	4·509	47·16		52·74		0·149
III Upper ...	4·623	46·35	47·66	53·33	52·10	0·208
Middle ...	4·723	47·82		52·18		0·114
Lower ...	4·858	48·81		50·80		0·265
IV Upper ...	4·974	49·15	49·37	50·28	50·12	0·211
Middle ...	5·138	49·70		49·85		0·223
Lower ...	5·312	49·27		50·24		0·243
Coccygeal—						
I Upper ...	5·486	52·60	52·70	46·65	46·74	0·278
Middle ...	5·622	52·93		46·86		0·313
Lower ...	5·726	52·58		46·71		0·352
II Upper ...	5·847	51·25	51·53	48·67	48·09	0·332
Middle ...	5·939	52·07		47·70		0·360
Lower ...	6·058	51·29		47·89		0·586
III Upper ...	6·174	50·90	48·62	47·60	50·44	0·898
Middle ...	6·278	47·58		52·03		0·676
Lower ...	6·331	47·37		51·68		0·956

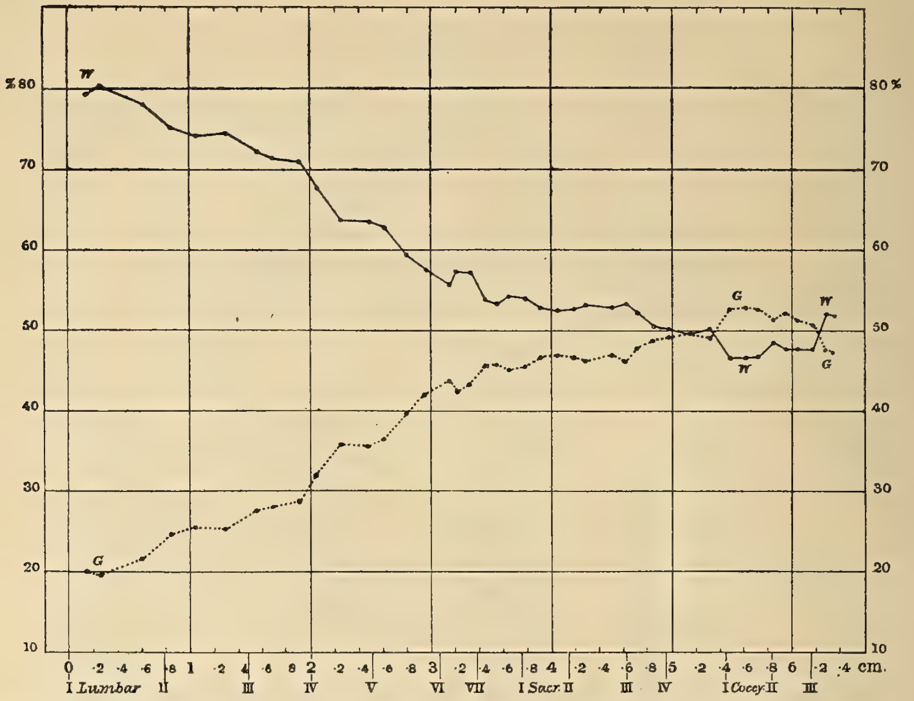

Chart III.—Curves showing the Percentage Value of the Grey and of the White Substance in the Cross-section of the Cord (Table VI).

The total area of each cross-section of the cord = 100. The ordinates denote percentage value, the abscissæ the length of the cord in centimetres measured from the uppermost section of the first lumbar region. The numbers (I, II, III, etc.) mark approximately the cephalic end of the region of each pair of spinal nerves.

Percentage of grey substance...... G, dotted line.
Percentage of white substance ... W, thin continuous line.

The percentage of grey substance in the cross-section of the cord (Chart III, G, Table VI) rises almost continuously from *ca.* 20 per cent. in the first lumbar region to 52 per cent. in the first coccygeal (maximum 52·9 per cent.). A slight fall in the percentage in the second coccygeal region is followed by a marked fall to 47 per cent. in the third coccygeal.

The percentage of white substance, on the other hand, falls almost continuously from *ca.* 80 per cent. in the first lumbar region to *ca.* 47 per cent. in the first coccygeal; and corresponding to the fall in percentage of grey substance, a slight rise in the second coccygeal is followed by a rapid rise to 51 per cent. in the third coccygeal region.

Thus, between the regions of the first lumbar and the first coccygeal nerve, there is an absolute rise in percentage of grey substance and a fall in that of white substance of 32.

The rise and fall in percentage of grey and white substance respectively is most rapid between the upper portion of the first lumbar region and the lower portion of the fifth lumbar region. Within these limits the rise and fall is most marked in the fourth and fifth lumbar regions, more particularly in the fifth. Through the first, second, and the greater part of the third sacral regions, there is very little variation in the percentage values.

In the middle portion of the fourth sacral region the percentage of grey and white substance is equal, and through the first, second, and part of the third coccygeal region the percentage of grey substance is higher than that of white.

The maximum percentage of grey substance and the lowest percentage of white substance is found in the first coccygeal region (*ca.* 53 per cent. and 47 per cent. respectively). The maximum percentage of white and the lowest percentage of grey substance is found in the first lumbar region (*ca.* 80 per cent. and 20 per cent. respectively).

(a) *Percentage of the Dorsal Columns (P) (Chart IV, Table VII).*

The percentage of the dorsal columns (P) to the cross-section of the cord falls from the first lumbar region to the end of the cord.

The dorsal columns constitute 22 per cent. of the cross-section of the cord in the first lumbar region, their percentage value being, approximately, the same in this as in both the sixth and twelfth dorsal (thoracic) regions (see Table VII).

Through the first lumbar region the percentage value of the dorsal columns is higher than that of the grey substance; while it is lower than this from the second lumbar region to the end of the cord.

Between the upper portion of the first lumbar region and the lower portion of the third coccygeal region there is a total fall of 14 in the percentage of the dorsal columns. The fall is most rapid between the upper portion of the first lumbar and that of the sixth lumbar region. It occurs chiefly in the third, fourth, and fifth, and more particularly in the fourth lumbar. From the sixth lumbar region to the end of the cord the fall is very gradual, the percentage value being approximately the same through the sacral regions.

(b) *Percentage of the Ventro-lateral Columns (A) (Chart IV, Table VII).*

The percentage of the ventro-lateral columns (A) to the cross-section of the cord falls from *ca.* 57 per cent. in the first lumbar region to *ca.* 36 per cent. in the first coccygeal, and rises from the middle portion of the latter region to 43 per cent. in the third coccygeal.

Percentage of the Dorsal and of the Ventro-lateral Columns to the Cross-sections of the Cord.

Table VII.—Percentage of the Dorsal and the Ventro-Lateral Columns to the Cross-Sections of the Cord. The total area of each cross-section of the cord = 100 (Charts IV, VII, and VIII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Percentage of the dorsal columns.	Mean percentage.	Percentage of the ventro-lateral columns.	Mean percentage.
Dorsal (thoracic)—					
VI	—	22 ·64	—	64 ·14	
XII	—	22 ·83	—	60 ·63	
Lumbar—					
I Upper ...	0 ·142	22 ·07	22 ·68	57 ·48	56 ·60
Middle ...	0 ·268	23 ·06		57 ·19	
Lower ...	0 ·608	22 ·92		55 ·14	
II Upper ...	0 ·846	22 ·67	22 ·15	52 ·55	52 ·51
Middle ...	1 ·058	22 ·03		52 ·22	
Lower ...	1 ·302	21 ·75		52 ·76	
III Upper ...	1 ·558	21 ·81	21 ·25	50 ·38	50 ·32
Middle ...	1 ·681	21 ·32		50 ·22	
Lower ...	1 ·918	20 ·63		50 ·35	
IV Upper ...	2 ·064	19 ·82	18 ·35	47 ·85	46 ·75
Middle ...	2 ·244	17 ·97		45 ·89	
Lower ...	2 ·484	17 ·26		46 ·52	
V Upper ...	2 ·608	16 ·74	15 ·72	46 ·15	44 ·34
Middle ...	2 ·794	15 ·40		44 ·17	
Lower ...	2 ·944	15 ·01		42 ·70	
VI Upper ...	3 ·154	13 ·45	13 ·59	42 ·52	42 ·94
Middle ...	3 ·205	13 ·78		43 ·63	
Lower ...	3 ·328	13 ·54		42 ·66	
VII Upper ...	3 ·450	13 ·27	13 ·00	40 ·60	40 ·79
Middle ...	3 ·557	13 ·09		40 ·50	
Lower ...	3 ·642	13 ·05		41 ·27	
Sacral—					
I Upper ...	3 ·782	12 ·18	11 ·69	41 ·91	41 ·58
Middle ...	3 ·906	11 ·70		41 ·28	
Lower ...	4 ·057	11 ·18		41 ·34	
II Upper ...	4 ·197	11 ·29	11 ·82	41 ·45	41 ·09
Middle ...	4 ·316	12 ·01		41 ·22	
Lower ...	4 ·509	12 ·14		40 ·60	
III Upper ...	4 ·623	11 ·67	12 ·03	41 ·67	40 ·07
Middle ...	4 ·723	12 ·62		39 ·56	
Lower ...	4 ·858	11 ·80		38 ·99	
IV Upper ...	4 ·974	11 ·55	11 ·64	38 ·73	38 ·49
Middle ...	5 ·138	11 ·49		38 ·36	
Lower ...	5 ·312	11 ·87		38 ·37	
Coccygeal—					
I Upper ...	5 ·486	10 ·04	10 ·11	36 ·62	36 ·63
Middle ...	5 ·622	10 ·67		36 ·19	
Lower ...	5 ·726	9 ·62		37 ·09	
II Upper ...	5 ·847	9 ·80	9 ·77	38 ·87	38 ·31
Middle ...	5 ·939	10 ·01		37 ·69	
Lower ...	6 ·058	9 ·51		38 ·38	
III Upper ...	6 ·174	8 ·08	7 ·92	39 ·52	42 ·52
Middle ...	6 ·278	7 ·54		44 ·49	
Lower ...	6 ·331	8 ·13		43 ·54	

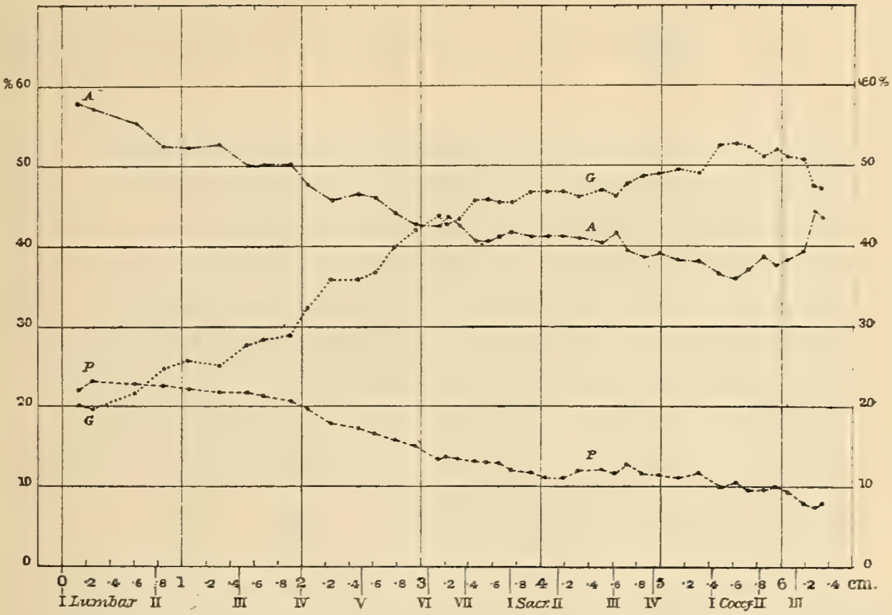

Chart IV.—Curves showing the Percentage Value of the Grey Substance and of the Dorsal and Ventro-lateral White Columns in the Cross-section of the Cord (Tables VI, VII).

The area of each cross-section of the cord = 100. The ordinates denote percentage value, the abscissæ the length of the cord in centimetres measured from the uppermost section of the first lumbar region. The numbers (I, II, III, etc.) mark approximately the cephalic end of the region of each pair of spinal nerves.

Percentage of grey substance	G, dotted line.
Percentage of dorsal white columns................	P, broken line.
Percentage of ventro-lateral white columns ...	A, line and dot.

The curve (A, Chart IV) shows alternate periods of falling value and a condition of equality or slight rise, especially between the first and seventh lumbar regions.*

Speaking generally, the fall in percentage is most rapid between the upper portion of the first lumbar and the lower portion of the fifth lumbar region. Taking the individual nerve regions, the most conspicuous fall occurs between the lower portion of the region of the third and that of the fifth lumbar nerve, it being most marked in the fifth.

The percentage value of the ventro-lateral columns (A) is equal to that of the grey substance (G) in the sixth lumbar region.

From the seventh lumbar to the third sacral region (upper portions) the percentage is practically the same.

This may indicate a segmental factor.

The rise, beginning in the middle portion of the first coccygeal, is continued through the second and third coccygeal regions. The percentage attained in the third coccygeal is equal to that in the fifth and sixth lumbar regions.

Hence it is seen that of the total fall of *ca.* 32 per cent. occurring in the percentage of the white substance (W) to the cross-sections of the cord, between the first lumbar and first coccygeal regions (upper portions), 11 is due to the fall in percentage of the dorsal columns (P), and 21 to that of the ventro-lateral columns (A), and that although the percentage value of both components of the white substance falls rapidly between the first and seventh lumbar regions, it is within these limits that the fall of the ventro-lateral columns so far exceeds that of the dorsal columns.

A further interesting point is the lack of correspondence between the fall of the ventro-lateral columns and that of the dorsal columns in the different nerve regions. This is especially noticeable between the first and seventh lumbar regions.

The comparatively slight rise seen in the percentage curve of the total white substance (W, Chart III) from the first to the third coccygeal region is accounted for by the continuance of the fall in percentage of the dorsal columns counteracting the rise in that of the ventro-lateral columns. The rise in the ventro-lateral columns (A) in the third coccygeal region is, however, more marked than the fall in the dorsal columns.

Comparison Between the Section Area and the Percentage Value of the Component Parts of the Cord.

For the purpose of comparing the section area with the percentage value of any one component part of the cord, the mean figures of both have been taken in the region of each spinal nerve (see Tables II to VII) and the curves plotted in the same chart.

Both the sectional area (G) and the percentage value (*g*) of the grey substance increase from the first to the fifth lumbar region. From the fifth lumbar to the third coccygeal region the sectional area decreases, whereas the percentage value increases to the first coccygeal, but decreases slightly in the second, and to a somewhat greater extent in the third coccygeal region.

(a) *Grey Substance.* (*Chart V, Tables II and VI.*)

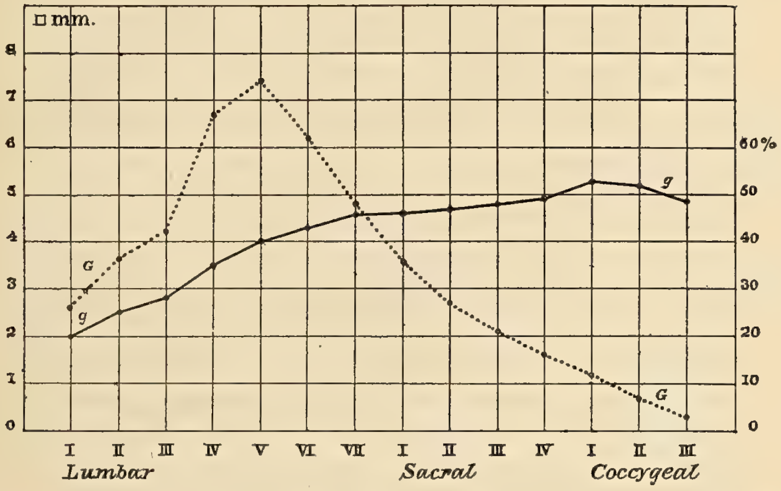

CHART V.—Curves showing the Mean Sectional Area (G, dotted line) and Percentage Value (g, continuous line) of the Grey Substance in the Cross-section of the Cord (Tables II, VI).

The ordinates on the left-hand side of the chart denote section area in square millimetres, and on the right-hand side percentage value. The abscissæ denote spinal nerve regions.

The sectional area of the white substance (W) increases from the first to the fourth lumbar region, whilst the percentage value (w) decreases.

Between the fourth and the seventh lumbar regions there is a decrease in both. From the seventh lumbar to the third sacral region the sectional area decreases, the percentage value remaining approximately the same.

From the third sacral to the first coccygeal there is a decrease in both, and from the first to the third coccygeal region the sectional area decreases, whilst the percentage value increases.

(b) *White Substance.* (*Chart VI, Tables III and VI.*)

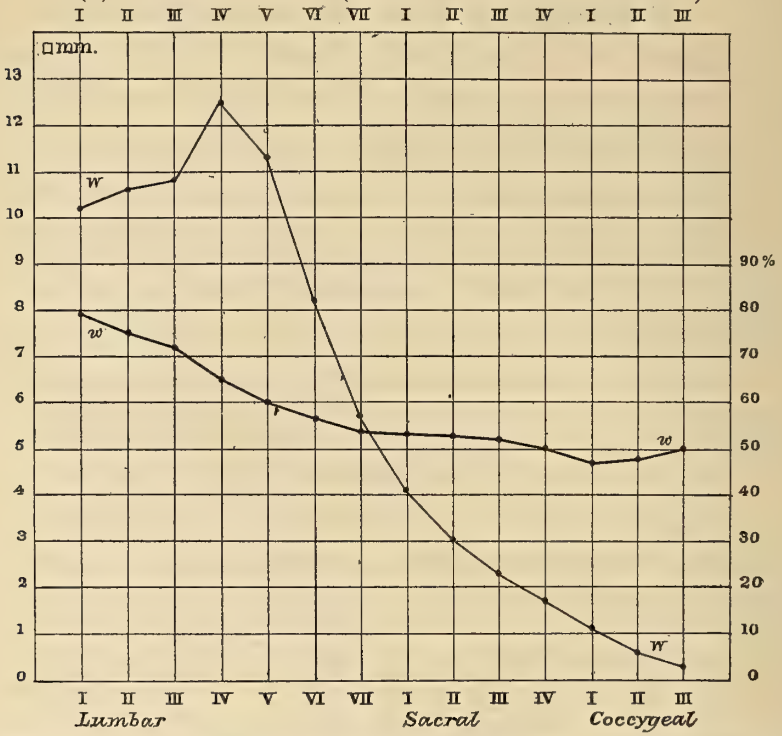

CHART VI.—Curves showing the Mean Sectional Area (W, thin continuous line) and Percentage Value (*w*, thick continuous line) of the White Substance in the Cross-section of the Cord (Tables III, VI).

The abscissæ denote spinal nerve regions, the value of the ordinates given on the left-hand side of the chart is for section area in square millimetres. For the percentage, the value of the ordinates is given on the right-hand side.

(c) *Dorsal Columns.* (*Chart VII, Tables IV and VII.*)

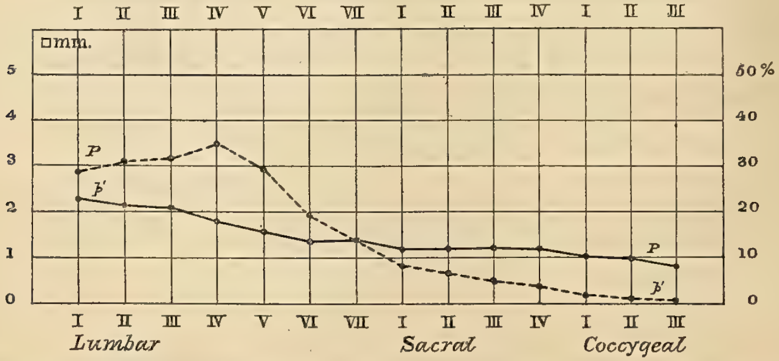

CHART VII.—Curves showing the Mean Sectional Area (P, dotted line), and the Mean Percentage Value (*p'*, continuous line) of the Dorsal Columns in the Cross-section of the Cord (Tables IV, VII).

The value of the ordinates given on the left-hand side of the chart indicates the section area in square millimetres, and on the right-hand side the percentage value. The abscissæ denote the spinal nerve regions.

The sectional area of the dorsal columns (P) increases from the first to the fourth lumbar region, the percentage value (p') at the same time decreases. From the fourth to the first sacral region there is a decrease in both; the sectional area continues to decrease from the first to the third sacral, whilst the percentage value increases slightly. From the third sacral to the third coccygeal region there is a decrease in both.

(d) *Ventro-Lateral Columns.* (*Chart VIII, Tables V and VII.*)

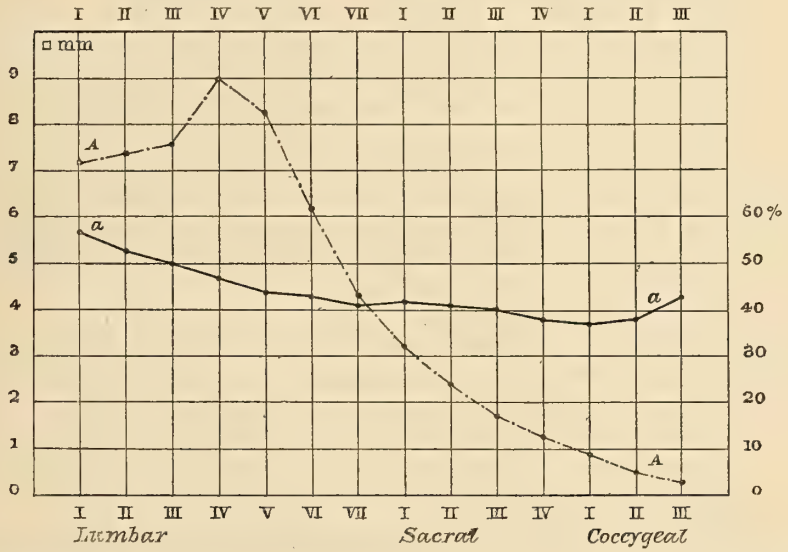

CHART VIII.—Curves showing the Mean Sectional Area (A, line and dot) and the Mean Percentage Value (a, continuous line) of the Ventro-lateral Columns in the Cross-section of the Cord (Tables V, VII).

For the section area in square millimetres the value of the ordinates is given on the left-hand side of the chart, and for the percentage on the right-hand side. The abscissæ denote the spinal nerve regions.

The sectional area of the ventro-lateral columns (A) increases from the first to the fourth lumbar region, whereas the percentage value (a) decreases.

From the fourth to the seventh lumbar there is a decrease in both. From the seventh lumbar to the first sacral the sectional area decreases and the percentage value increases.

From the second sacral to the first coccygeal region there is a decrease in both, and while the sectional area continues to decrease from the first to the third coccygeal region the percentage value increases, and to the greatest extent in the third coccygeal region.

A 3. *Ratio of the Total White Substance, and of the Dorsal and of the Ventro-Lateral Columns to the Grey Substance.*

Table VIII.—Ratio of the Total White Substance, and of the Dorsal and of the Ventro-lateral White Columns, to the Grey Substance taken as Unity in each Cross Section of the Cord (Chart IX).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	White substance. / Grey substance.	Mean.	Dorsal columns. / Grey substance.	Mean.	Ventro-lateral columns. / Grey substance.	Mean.
Dorsal (thoracic)—							
VI	—	6 ·78		1 ·77		5 ·01	
XII	—	5 ·06		1 ·38		3 ·67	
Lumbar—							
I Upper	0 ·142	3 ·96	3 ·89	1 ·09	1 ·11	2 ·86	2 ·78
I Middle	0 ·268	4 ·08		1 ·17		2 ·91	
I Lower	0 ·608	3 ·62		1 ·06		2 ·56	
II Upper	0 ·846	3 ·06	2 ·98	0 ·92	0 ·88	2 ·14	2 ·10
II Middle	1 ·058	2 ·90		0 ·86		2 ·04	
II Lower	1 ·302	2 ·98		0 ·87		2 ·11	
III Upper	1 ·558	2 ·62	2 ·49	0 ·78	0 ·75	1 ·72	1 ·75
III Middle	1 ·681	2 ·52		0 ·75		1 ·77	
III Lower	1 ·918	2 ·47		0 ·72		1 ·75	
IV Upper	2 ·064	2 ·10	1 ·88	0 ·61	0 ·53	1 ·49	1 ·35
IV Middle	2 ·244	1 ·77		0 ·50		1 ·27	
IV Lower	2 ·484	1 ·77		0 ·48		1 ·29	
V Upper	2 ·608	1 ·72	1 ·53	0 ·46	0 ·40	1 ·26	1 ·13
V Middle	2 ·794	1 ·49		0 ·39		1 ·10	
V Lower	2 ·944	1 ·37		0 ·36		1 ·01	
VI Upper	3 ·154	1 ·28	1 ·31	0 ·31	0 ·31	0 ·97	0 ·99
VI Middle	3 ·205	1 ·35		0 ·32		1 ·03	
VI Lower	3 ·328	1 ·29		0 ·31		0 ·98	
VII Upper	3 ·450	1 ·18	1 ·18	0 ·29	0 ·29	0 ·89	0 ·89
VII Middle	3 ·557	1 ·17		0 ·28		0 ·88	
VII Lower	3 ·642	1 ·20		0 ·29		0 ·91	

Sacral—							
I Upper …………	3 ·782	1 ·19	1 ·15	0 ·27	0 ·25	0 ·92	0 ·90
I Middle …………	3 ·906	1 ·14		0 ·25		0 ·89	
I Lower …………	4 ·057	1 ·11		0 ·23		0 ·88	
II Upper …………	4 ·197	1 ·12	1 ·13	0 ·24	0 ·25	0 ·88	0 ·88
II Middle …………	4 ·316	1 ·15		0 ·26		0 ·89	
II Lower …………	4 ·509	1 ·12		0 ·26		0 ·86	
III Upper …………	4 ·623	1 ·14	1 ·09	0 ·25	0 ·25	0 ·89	0 ·84
III Middle …………	4 ·723	1 ·09		0 ·26		0 ·83	
III Lower …………	4 ·858	1 ·04		0 ·24		0 ·79	
IV Upper …………	4 ·974	1 ·02	1 ·01	0 ·23	0 ·23	0 ·79	0 ·78
IV Middle …………	5 ·138	1 ·00		0 ·23		0 ·77	
IV Lower …………	5 ·312	1 ·02		0 ·24		0 ·78	
Coccygeal—							
I Upper …………	5 ·486	0 ·89	0 ·89	0 ·19	0 ·20	0 ·69	0 ·70
I Middle …………	5 ·622	0 ·89		0 ·21		0 ·69	
I Lower …………	5 ·726	0 ·89		0 ·19		0 ·71	
II Upper …………	5 ·847	0 ·96	0 ·94	0 ·19	0 ·19	0 ·77	0 ·75
II Middle …………	5 ·939	0 ·91		0 ·19		0 ·72	
II Lower …………	6 ·058	0 ·93		0 ·18		0 ·75	
III Upper …………	6 ·174	0 ·94	1 ·04	0 ·16	0 ·17	0 ·78	0 ·88
III Middle …………	6 ·278	1 ·09		0 ·16		0 ·94	
III Lower …………	6 ·331	1 ·09		0 ·17		0 ·92	

The ratio $\frac{\text{white substance}}{\text{grey substance}}$ having been determined for the upper, middle, and lower portions of the region of each spinal nerve, a mean of the three ratios has been employed in the curve to represent the ratio of the region.*

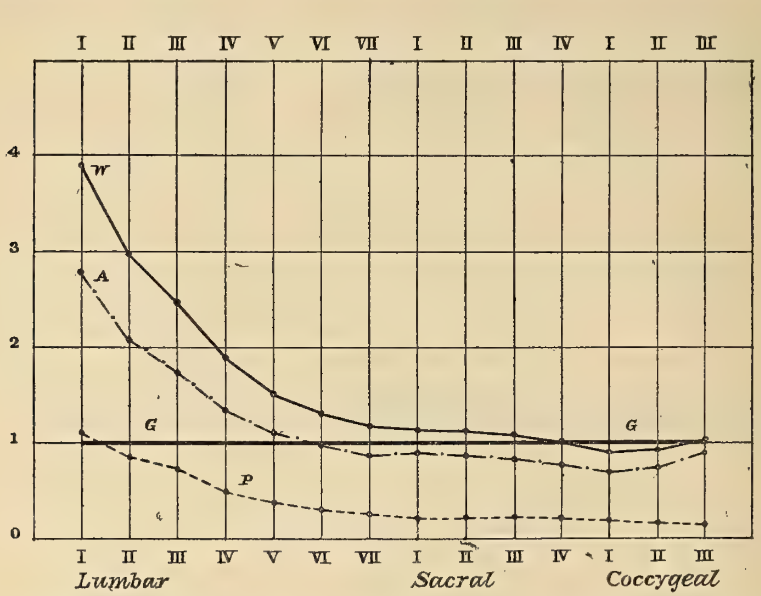

Chart IX.—Curves showing the Ratio of the total White Substance (W, continuous line), of the Dorsal White Columns (P, broken line), and of the Ventro-lateral White Columns (A, line and dot) to the Grey Substance (G).

The grey substance is taken as unity in each cross-section of the cord. Mean results are plotted (Table VIII). The abscissæ denote the spinal nerve regions, the ordinates the ratio at the corresponding regions.

(*a*) Ratio $\frac{\text{white substance (W)}}{\text{grey substance (G)}}$ Chart IX, Table VIII.

The ratio $\frac{\text{total white substance (W)}}{\text{grey substance (G)}}$ decreases continuously from the first lumbar to the first coccygeal region. From the first to the third coccygeal region a very slight increase occurs.

The decrease in the ratio is very rapid from the first to the seventh lumbar region, and is most conspicuous in the first three lumbar regions. In the seventh lumbar and through the first three sacral regions the decrease in the ratio is scarcely perceptible. In the fourth sacral region the ratio is as 1 : 1, and while in the first and second coccygeal regions it is slightly less, equality is again reached in the third coccygeal region.

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* The same remark applies to all subsequent curves illustrating ratio.

(*b*) Ratio $\frac{\text{dorsal columns (P)}}{\text{grey substance (G)}}$ (Chart IX, Table VIII).

The curve (P) representing the ratio $\frac{\text{dorsal columns}}{\text{grey substance}}$ differs from that of the ventro-lateral columns (A) in the total fall being much less pronounced.

The ratio starting as only slightly more than 1 : 1 in the first lumbar region falls gradually, and is less than 1 : 1 in the second lumbar region, and throughout the remainder of the cord. Through the first three sacral regions the ratio is approximately the same. The grey substance being taken as unity, the index of the fall from the first lumbar to the third coccygeal region is only 0·95, the principal decrease occurring in the lumbar regions (0·82) and chiefly in those of the first five lumbar nerves.

(*c*) Ratio $\frac{\text{ventro-lateral columns (A)}}{\text{grey substance (G)}}$ (Chart IX, Table VIII).

The curve (A) indicating the ratio $\frac{\text{ventro-lateral columns}}{\text{grey substance}}$ resembles in its general configuration the curve (W) denoting the ratio $\frac{\text{total white substance}}{\text{grey substance}}$.

The ratio decreases rapidly from the first to the seventh lumbar region, the decrease being most marked in the regions of the first five lumbar nerves. In the seventh lumbar and first and second sacral regions the ratio is almost the same. A slight decrease from the third sacral to the first coccygeal region is followed by a slight increase in the lower coccygeal regions, the ratio being approximately the same in the third coccygeal as in the seventh lumbar region.

Although the absolute fall in the ratio $\frac{\text{ventro-lateral columns}}{\text{grey substance}}$ is much greater than that in the ratio $\frac{\text{dorsal columns}}{\text{grey substance}}$, relatively the variation of the latter is considerably greater.

This is seen in Chart X, where the ordinates indicate the relative values of the ratios, when the ratio for the first lumbar region is in each case taken as 10 (Table IX).

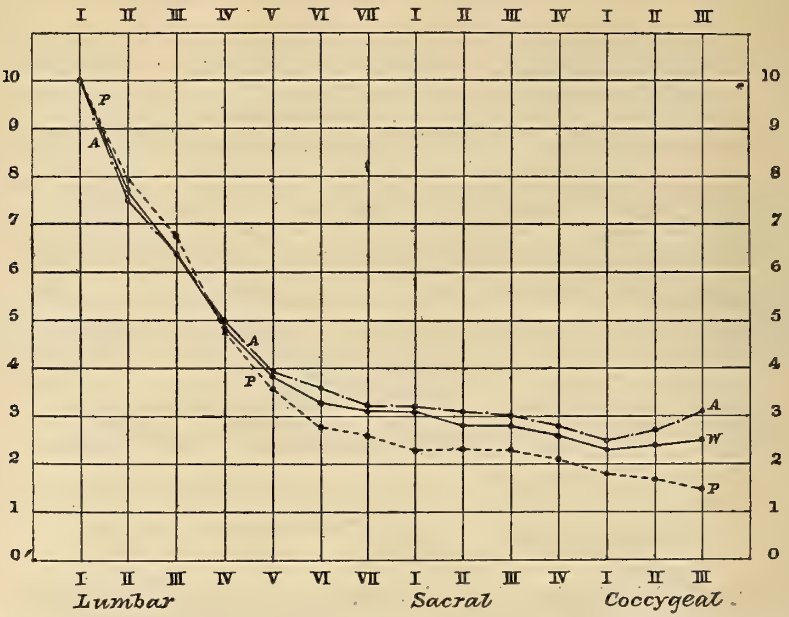

CHART X.—Curves showing the Relative Values of the Ratios $\frac{\text{Total White Substance}}{\text{Grey Substance}}$ (W, continuous line), $\frac{\text{Dorsal Columns}}{\text{Grey Substance}}$ (P, broken line), $\frac{\text{Ventro-lateral Columns}}{\text{Grey Substance}}$ (A, line and dot) (Table IX).

The ratio in the first lumbar region is in each case taken as 10 (see Table VIII). The abscissæ denote spinal nerve regions, the ordinates relative value at corresponding regions.

Table IX.—Relative Values of the Ratios $\frac{\text{Total White Substance}}{\text{Grey Substance}}$, $\frac{\text{Dorsal Columns}}{\text{Grey Substance}}$, and $\frac{\text{Ventro-lateral Columns}}{\text{Grey Substance}}$. The ratio in the I Lumbar region is in each case taken as 10 (Chart X).

Region of cord.	Total white substance.	Dorsal columns.	Ventro-lateral columns.
Lumbar—			
I …………	10·0	10·0	10·0
II …………	7·7	7·9	7·5
III …………	6·4	6·8	6·4
IV …………	4·9	4·8	5·0
V …………	3·8	3·6	3·9
VI …………	3·3	2·8	3·6
VII …………	3·1	2·6	3·2
Sacral—			
I …………	3·1	2·3	3·2
II …………	2·8	2·3	3·1
III …………	2·8	2·3	3·0
IV …………	2·6	2·1	2·8
Coccygeal—			
I …………	2·3	1·8	2·5
II …………	2·4	1·7	2·7
III …………	2·5	1·5	3·1

A4. *Ratio of the Dorsal and of the Ventro-lateral Columns to the White Substance as a Whole.*

Table X.—Ratio of the Dorsal Columns and of the Ventro-lateral Columns to the Total White Substance taken as Unity in each Cross-section of the Cord (Chart XI).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Dorsal columns / Total white substance.	Mean.	Ventro-lateral columns / Total white substance.	Mean.
Dorsal (thoracic)—					
VI................	—	0·261	—	0·769	
XII................	—	0·274	—	0·726	
Lumbar—					
I Upper ...	0·142	0·278	0·286	0·722	0·714
Middle...	0·268	0·287		0·713	
Lower ...	0·608	0·294		0·706	
II Upper ...	0·846	0·301	0·297	0·699	0·703
Middle...	1·058	0·297		0·703	
Lower ...	1·302	0·292		0·708	
III Upper ...	1·558	0·301	0·297	0·698	0·703
Middle...	1·681	0·298		0·702	
Lower ...	1·918	0·291		0·709	
IV Upper ...	2·064	0·293	0·282	0·707	0·718
Middle...	2·244	0·281		0·719	
Lower ...	2·484	0·271		0·729	
V Upper ...	2·608	0·266	0·262	0·734	0·738
Middle...	2·794	0·258		0·741	
Lower ...	2·944	0·260		0·740	
VI Upper ...	3·154	0·240	0·240	0·760	0·760
Middle...	3·205	0·240		0·760	
Lower ...	3·328	0·241		0·759	
VII Upper ...	3·450	0·246	0·244	0·754	0·756
Middle...	3·557	0·244		0·756	
Lower ...	3·642	0·240		0·760	
Sacral—					
I Upper ...	3·782	0·225	0·220	0·774	0·780
Middle...	3·906	0·221		0·779	
Lower ...	4·057	0·213		0·787	
II Upper ...	4·197	0·214	0·224	0·785	0·776
Middle...	4·316	0·226		0·774	
Lower ...	4·509	0·231		0·769	
III Upper ...	4·623	0·219	0·231	0·781	0·769
Middle...	4·723	0·242		0·758	
Lower ...	4·858	0·232		0·768	
IV Upper ...	4·974	0·230	0·232	0·770	0·768
Middle...	5·138	0·231		0·769	
Lower ...	5·312	0·236		0·764	
Coccygeal—					
I Upper ...	5·486	0·215	0·216	0·785	0·784
Middle...	5·622	0·228		0·772	
Lower ...	5·726	0·206		0·794	
II Upper ...	5·847	0·201	0·203	0·798	0·796
Middle...	5·939	0·210		0·790	
Lower ...	6·058	0·199		0·801	
III Upper ...	6·174	0·170	0·157	0·830	0·843
Middle...	6·278	0·145		0·855	
Lower ...	6·331	0·157		0·843	

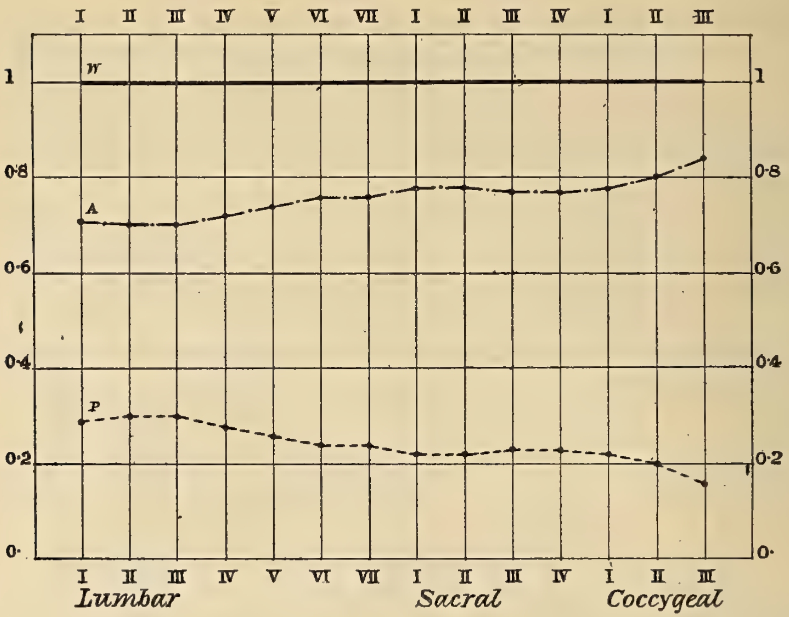

CHART XI.—Curves showing the Ratio of the Dorsal Columns (P, broken line) and of the Ventro-lateral Columns (A, line and dot) to the White Substance as a whole (W).

The white substance in each cross-section of the cord is taken as unity. Mean figures are plotted (Table X). The abscissæ denote the spinal nerve regions, the ordinates the ratio at the corresponding regions.

The ratio $\frac{\text{dorsal columns (P)}}{\text{white substance (W)}}$ (Chart XI, Table X) increases slightly from the first lumbar region to the second, and remains the same in the third. It decreases from the fourth lumbar to the first sacral region, being the same in both the sixth and seventh lumbar. Through the sacral regions and the first coccygeal the ratio varies very little; it again decreases in the lower coccygeal regions.

The two curves being complementary, the above remarks apply inversely to the ratio $\frac{\text{ventro-lateral columns (A)}}{\text{white substance (W)}}$ (Chart XI, Table X).

A5. *Ratio of the Ventro-lateral Columns to the Dorsal Columns.*

Table XI.—Ratio of the Ventro-lateral Columns to the Dorsal Columns taken as Unity in each Cross-section of the Cord (Chart XII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	$\frac{\text{Ventro-lateral columns.}}{\text{Dorsal columns.}}$	Mean.
Dorsal—			
VI	—	2·83	
XII	—	2·56	
Lumbar—			
I Upper	0·142	2·61	2·50
I Middle	0·268	2·48	
I Lower	0·608	2·40	
II Upper	0·846	2·32	2·37
II Middle	1·058	2·37	
II Lower	1·302	2·43	
III Upper	1·558	2·31	2·37
III Middle	1·681	2·36	
III Lower	1·918	2·41	
IV Upper	2·064	2·42	2·56
IV Middle	2·244	2·55	
IV Lower	2·484	2·70	
V Upper	2·608	2·75	2·82
V Middle	2·794	2·86	
V Lower	2·944	2·84	
VI Upper	3·154	3·16	3·16
VI Middle	3·205	3·16	
VI Lower	3·328	3·15	
VII Upper	3·450	3·05	3·10
VII Middle	3·557	3·09	
VII Lower	3·642	3·16	
Sacral—			
I Upper	3·782	3·44	3·55
I Middle	3·906	3·51	
I Lower	4·057	3·70	
II Upper	4·197	3·67	3·48
II Middle	4·316	3·43	
II Lower	4·509	3·34	
III Upper	4·623	3·57	3·34
III Middle	4·723	3·14	
III Lower	4·858	3·30	
IV Upper	4·974	3·37	3·29
IV Middle	5·138	3·31	
IV Lower	5·312	3·19	
Coccygeal—			
I Upper	5·486	3·65	3·63
I Middle	5·622	3·39	
I Lower	5·726	3·85	
II Upper	5·847	3·97	3·93
II Middle	5·939	3·77	
II Lower	6·058	4·04	
III Upper	6·174	4·89	5·38
III Middle	6·278	5·90	
III Lower	6·331	5·35	

The ratio $\frac{\text{ventro-lateral columns (A)}}{\text{dorsal columns (P)}}$ (Chart XII, Table XI) falls slightly from the first to the second lumbar region, is constant in the third, and increases from the fourth to the first sacral region, a slight fall being noticed in the seventh lumbar. The ratio decreases in the second, third, and fourth sacral regions, but increases again through the coccygeal regions, the increase being very marked in the third.

Comparing the ratio of the two columns in different regions with that in the first lumbar, it is seen that the ratio is 1·4 and 1·6 times as great in the first sacral and second coccygeal regions respectively, and becomes 2·1 times as great in the third coccygeal.

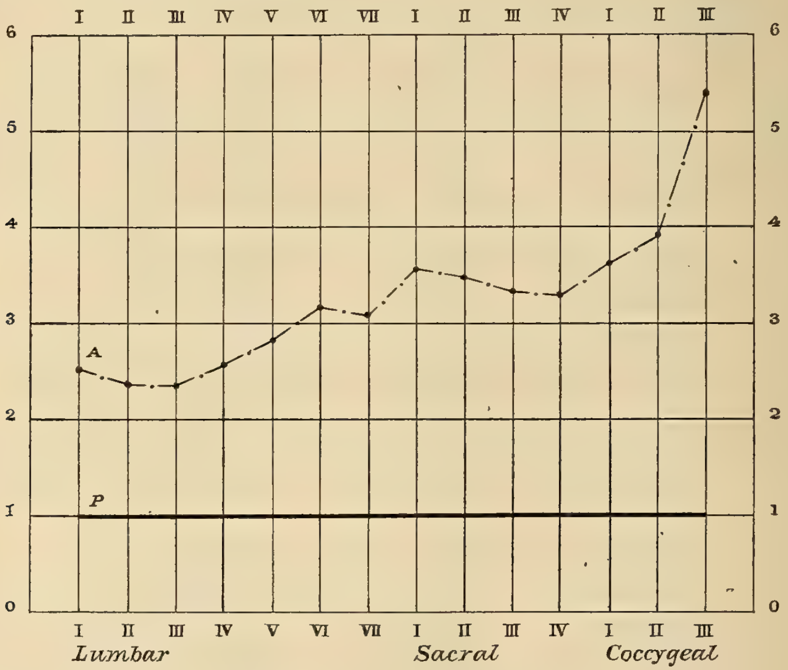

Chart XII.—Curves showing the Ratio of the Ventro-lateral Columns (A, line and dot) to the Dorsal Columns (P).

The dorsal columns taken as unity in each cross-section of the cord. Mean results are plotted (Table XI). The abscissæ denote spinal nerve regions, the ordinates the ratio at the corresponding regions.

B1. *Section Area of the Dorsal and of the Ventral Horns of the Grey Substance in the Region of each Spinal Nerve.*

A remarkable difference is observable in the form of the two curves representing the section areas of the dorsal and the ventral horns of the grey substance.*

The curve indicating the dorsal horns (D) (Chart XIII) is comparatively flat throughout the portion of the cord examined, whilst that denoting the ventral horns (V), with its marked rise and fall, resembles in every way the curve illustrating the section area of the grey substance as a whole.

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* For definition of terms "dorsal" and "ventral horns" see p. 90.

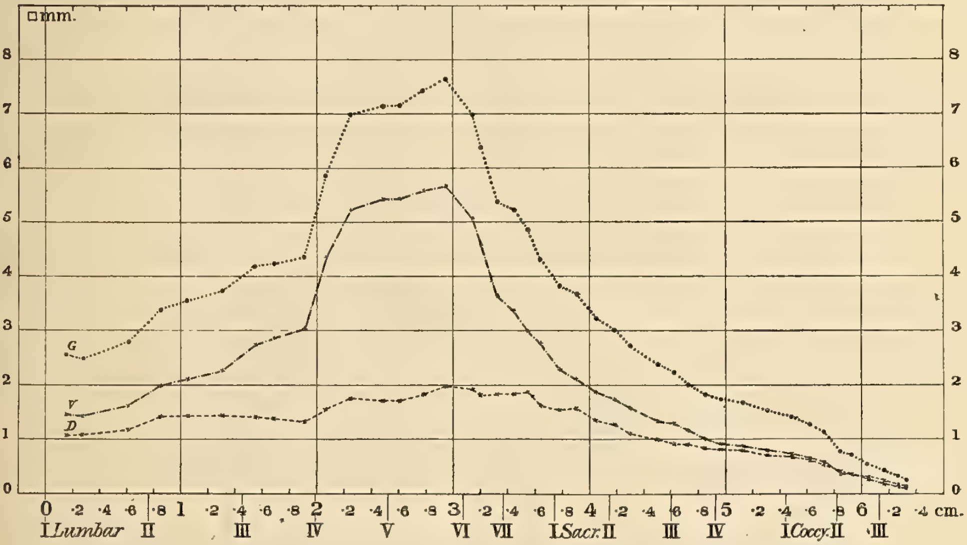


Chart XIII.—Curves showing the Section Areas of the Grey Substance and of the Dorsal and the Ventral Horns of the same (Tables II, XII, XIII).

The ordinates denote section area in square millimetres, and the abscissæ the length of the cord in centimetres measured from the uppermost section of the first lumbar region. The Roman figures mark approximately the cephalic end of the region of each pair of spinal nerves.

Grey substance as a whole......	G, dotted line.
Dorsal horns........................	D, interrupted line and cross.
Ventral horns	V, line, dot, and cross.

(a) *Section Area of the Dorsal Horns.*

The section area of the dorsal horns (Chart XIII D, Table XII) increases from the first to the fifth lumbar region, and decreases from the lower portion of the fifth to the end of the cord. The actual increase between the first and fifth lumbar regions amounts to 0·87 sq. mm. (1·1—1·97 sq. mm.). The section area at its maximum (V L, lower) is thus almost twice as great as in the first lumbar region.

It will be observed that the maximum is reached in the same region, as the maximum section area of the grey substance (G).

Both the increase and decrease in the section area of the dorsal horns take place gradually, but the decrease is more rapid than the increase. The section area varies very little in the first lumbar region, it becomes rather larger in the upper portion of the second, and remains much the same through this and the third. The chief increase occurs between the lower portion of the third and that of the fifth lumbar region, and is most marked in the fifth.

Table XII.—Section Area of the Dorsal Horns (Chart XIII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	2·28	0·570	
XII	—	3·16	0·790	
Lumbar—				
I Upper	0·142	4·38	1·095	1·108
I Middle	0·268	4·28	1·070	
I Lower	0·608	4·68	1·170	
II Upper	0·846	5·68	1·420	1·438
II Middle	1·058	5·76	1·440	
II Lower	1·302	5·82	1·455	
III Upper	1·558	5·64	1·410	1·365
III Middle	1·681	5·50	1·375	
III Lower	1·918	5·24	1·310	
IV Upper	2·064	6·02	1·505	1·657
IV Middle	2·244	7·02	1·755	
IV Lower	2·484	6·85	1·712	
V Upper	2·608	6·82	1·705	1·840
V Middle	2·794	7·38	1·845	
V Lower	2·944	7·88	1·970	
VI Upper	3·154	7·64	1·910	1·812
VI Middle	3·205	7·24	1·810	
VI Lower	3·328	6·86	1·715	
VII Upper	3·450	7·34	1·835	1·781
VII Middle	3·557	7·49	1·873	
VII Lower	3·642	6·54	1·635	
Sacral—				
I Upper	3·782	6·16	1·540	1·492
I Middle	3·906	6·32	1·580	
I Lower	4·057	5·42	1·355	
II Upper	4·197	5·14	1·285	1·412
II Middle	4·316	4·46	1·115	
II Lower	4·509	4·10	1·025	
III Upper	4·623	3·74	0·9350	0·8950
III Middle	4·723	3·64	0·9100	
III Lower	4·858	3·36	0·8400	
IV Upper	4·974	3·28	0·8200	0·7766
IV Middle	5·138	3·18	0·7950	
IV Lower	5·312	2·86	0·7150	
Coccygeal—				
I Upper	5·486	2·76	0·6900	0·6166
I Middle	5·622	2·46	0·6150	
I Lower	5·726	2·18	0·5450	
II Upper	5·847	6·40	0·4000	0·3502
II Middle	5·939	5·89	0·3681	
II Lower	6·058	4·52	0·2825	
III Upper	6·174	0·96	0·2400	0·1868
III Middle	6·278	2·86	0·1787	
III Lower	6·331	0·59	0·1475	

The decrease in section area is very slight between the fifth and the middle portion of the seventh lumbar region. It is accentuated between the seventh lumbar (middle portion) and the third sacral region, and, with the exception of being marked between the first and second coccygeal regions, is continued in a regular manner to the end of the cord.

In the first coccygeal region the section area of the dorsal horns (D) is almost equal to that of the ventral horns (V), and is slightly greater in the second and third coccygeal regions.

(b) *Section Area of the Ventral Horns.*

The section area of the ventral horns (Chart XIII V, Table XIII) increases from the first to the lower portion of the fifth lumbar region, where the maximum (5·68 sq. mm.) is reached. The maximum is thus found in the same region (VL) as the maximum section areas of the dorsal horns (D), and of the grey substance as a whole (G).

The section area of the ventral horns (V) decreases from the fifth lumbar region to the end of the cord.

The increase in section area is very gradual from the first to the lower part of the third lumbar region, but becomes very marked between the latter and the lower part of the fifth lumbar region, three-fifths of the whole increase occurring here. The most rapid increase is between the third and fourth and in the fourth lumbar region. Throughout the fifth lumbar the section area is nearly four times as great as that in the first lumbar region.

The decrease in section area is more rapid than the increase, and is very marked between the lower portion of the fifth and that of the sixth lumbar region. From the seventh lumbar region to the end of the cord, the decrease continues in a more regular manner and is least marked between the third sacral and second coccygeal regions.

The section area of the ventral horns (V) in the seventh lumbar region (*ca.* 3 sq. mm.) is much the same as that in the third lumbar region, and in the second sacral (*ca.* 1·5 sq. mm.) it is much the same as in the first lumbar region.

Table XIII.—Section Area of the Ventral Horns (Chart XIII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Planimeter measurement in sq. cm.	Actual area as calculated in sq. mm.	Mean.
Dorsal (thoracic)—				
VI	—	2·70	0·675	
XII	—	4·40	1·100	
Lumbar—				
I Upper	0·142	5·84	1·460	1·495
Middle	0·268	5·66	1·415	
Lower	0·608	6·44	1·610	
II Upper	0·846	7·90	1·975	2·115
Middle	1·058	8·44	2·210	
Lower	1·302	9·04	2·260	
III Upper	1·558	11·00	2·750	2·875
Middle	1·681	11·40	2·850	
Lower	1·918	12·10	3·025	
IV Upper	2·064	17·40	4·350	4·993
Middle	2·244	20·84	5·210	
Lower	2·484	21·68	5·420	
V Upper	2·608	21·78	5·445	5·570
Middle	2·794	22·36	5·590	
Lower	2·944	22·70	5·675	
VI Upper	3·154	20·26	5·065	4·423
Middle	3·205	18·25	4·563	
Lower	3·328	14·56	3·640	
VII Upper	3·450	13·50	3·375	3·028
Middle	3·557	11·96	2·990	
Lower	3·642	10·88	2·720	
Sacral—				
I Upper	3·782	9·12	2·280	2·082
Middle	3·906	8·42	2·105	
Lower	4·057	7·44	1·860	
II Upper	4·197	7·00	1·750	1·555
Middle	4·316	6·28	1·570	
Lower	4·509	5·38	1·345	
III Upper	4·623	5·16	1·290	1·155
Middle	4·723	4·70	1·175	
Lower	4·858	4·00	1·000	
IV Upper	4·974	3·70	0·9250	0·8650
Middle	5·138	3·48	0·8700	
Lower	5·312	3·20	0·8000	
Coccygeal—				
I Upper	5·486	2·90	0·7250	0·6500
Middle	5·622	2·60	0·6500	
Lower	5·726	2·30	0·5750	
II Upper	5·847	5·94	0·3712	0·3295
Middle	5·939	5·66	0·3537	
Lower	6·058	4·22	0·2637	
III Upper	6·174	0·74	0·1850	0·1379
Middle	6·278	2·06	0·1287	
Lower	6·331	0·40	0·1000	

B2. *Percentage of the Ventral and of the Dorsal Horns to the Total Grey Substance in the Cross-section of the Cord.*

Table XIV.—Percentage Values of the Dorsal and of the Ventral Horns in the Total Grey Substance. The total area of the grey substance in each cross-section of the cord = 100 (Chart XIV).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Percentage of dorsal horns.	Mean.	Percentage of ventral horns.	Mean.
Dorsal (thoracic)—					
VI	—	45·78	—	54·22	
XII	—	41·80	—	58·20	
Lumbar—					
I Upper	0·142	42·86	42·67	57·14	57·33
Middle	0·268	43·06		56·94	
Lower	0·608	42·09		57·91	
II Upper	0·846	41·83	40·52	58·17	59·48
Middle	1·058	40·56		59·44	
Lower	1·302	39·17		60·83	
III Upper	1·558	33·89	32·22	66·11	67·78
Middle	1·681	32·54		67·46	
Lower	1·918	30·22		69·78	
IV Upper	2·064	25·70	24·97	74·30	75·02
Middle	2·244	25·20		74·80	
Lower	2·484	24·00		75·96	
V Upper	2·608	23·85	24·81	76·15	75·19
Middle	2·794	24·82		75·18	
Lower	2·944	25·77		74·23	
VI Upper	3·154	27·38	29·27	72·62	70·73
Middle	3·205	28·40		71·60	
Lower	3·328	32·03		67·97	
VII Upper	3·450	35·22	37·09	64·78	62·91
Middle	3·557	38·51		61·49	
Lower	3·642	37·54		62·46	
Sacral—					
I Upper	3·782	40·31	41·78	59·69	58·22
Middle	3·906	42·88		57·12	
Lower	4·057	42·15		57·85	
II Upper	4·197	42·34	42·38	57·66	57·62
Middle	4·316	41·53		58·47	
Lower	4·509	43·25		56·75	
III Upper	4·623	42·02	43·77	57·98	56·23
Middle	4·723	43·65		56·35	
Lower	4·858	45·65		54·35	
IV Upper	4·974	46·99	47·31	53·01	52·69
Middle	5·138	47·75		52·25	
Lower	5·312	47·19		52·81	
Coccygeal—					
I Upper	5·486	48·76	48·81	51·24	51·19
Middle	5·622	49·01		50·99	
Lower	5·726	48·66		51·34	
II Upper	5·847	51·86	51·66	48·14	48·34
Middle	5·939	51·38		48·62	
Lower	6·058	51·72		48·28	
III Upper	6·174	56·47	58·07	43·53	41·93
Middle	6·278	58·13		41·87	
Lower	6·331	59·60		40·40	

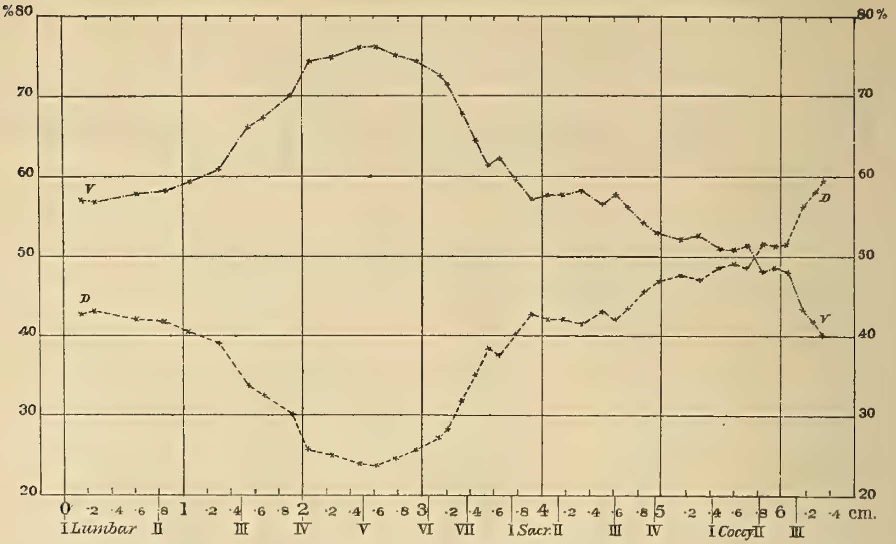


CHART XIV.—Curves showing the Percentage Values of the Ventral and of the Dorsal Horns in the Total Grey Substance.

The total area of the grey substance in each cross-section of the cord is taken as 100. The ordinates denote percentage value, the abscissæ the length of the cord in centimetres measured from the uppermost section of the first lumbar region.

Percentage of ventral horns...... V, line, dot, and cross.
Percentage of dorsal horns ...... D, broken line and cross.

The percentage of the ventral horns (V) (Chart XIV, Table XIV) to the total grey substance in the cross-section of the cord increases to a very marked extent between the first lumbar and the upper portion of the fifth lumbar region, where the maximum (76 per cent.) is reached. The absolute rise is 19 per cent. The percentage decreases from the upper portion of the fifth lumbar region to the end of the cord, the absolute fall to the third coccygeal region amounting to *ca.* 36 per cent.

Inversely, the percentage of the dorsal horns decreases from the first to the fifth lumbar region, and increases from the latter region to the end of the cord, the maximum (59 per cent.) being reached in the region of the third coccygeal nerve.

The rise in the percentage of the ventral horns is very rapid between the lower portion of the second lumbar region and the upper portion of the fourth.

The fall in percentage is most marked between the fifth lumbar and the first sacral region (middle portion), and occurs chiefly in the sixth lumbar. It again becomes marked between the second and third coccygeal, and in the latter region.

Inversely, the same remarks apply to the curve representing the percentage of the dorsal horns to the total grey substance in the cross-section of the cord.

B3. *Ratio of the Ventral to the Dorsal Horns.*

Table XV.—Ratio of the Ventral to the Dorsal Horns taken as Unity in each Cross-section of the Cord (Chart XV).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Ventral horns. / Dorsal horns.	Mean.
Dorsal (thoracic)—			
VI	—	1·84	
XII	—	1·39	
Lumbar—			
I Upper	0·142	1·33	1·35
Middle	0·268	1·33	
Lower	0·608	1·38	
II Upper	0·846	1·39	1·47
Middle	1·058	1·47	
Lower	1·302	1·55	
III Upper	1·558	1·95	2·11
Middle	1·681	2·07	
Lower	1·918	2·31	
IV Upper	2·064	2·89	3·01
Middle	2·244	2·97	
Lower	2·484	3·16	
V Upper	2·608	3·19	3·03
Middle	2·794	3·03	
Lower	2·944	2·88	
VI Upper	3·154	2·65	2·43
Middle	3·205	2·52	
Lower	3·328	2·12	
VII Upper	3·450	1·84	1·70
Middle	3·557	1·60	
Lower	3·642	1·66	
Sacral—			
I Upper	3·782	1·48	1·40
Middle	3·906	1·33	
Lower	4·057	1·37	
II Upper	4·197	1·36	1·36
Middle	4·316	1·41	
Lower	4·509	1·31	
III Upper	4·623	1·38	1·29
Middle	4·723	1·29	
Lower	4·858	1·19	
IV Upper	4·974	1·13	1·11
Middle	5·138	1·09	
Lower	5·312	1·12	
Coccygeal—			
I Upper	5·486	1·05	1·05
Middle	5·622	1·06	
Lower	5·726	1·05	
II Upper	5·847	0·928	0·933
Middle	5·939	0·961	
Lower	6·058	0·911	
III Upper	6·174	0·771	0·723
Middle	6·278	0·720	
Lower	6·331	0·678	

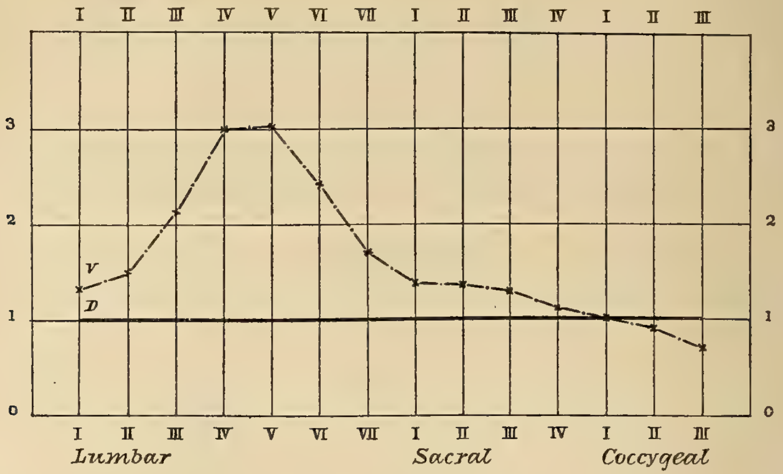


CHART XV.—Curve showing the Ratio of the Ventral Horns (V, line, dot, and cross) to the Dorsal Horns (D).

The dorsal horns taken as unity in each cross-section of the cord. Mean results are plotted (Table XV). The abscissæ denote spinal nerve regions, the ordinates ratio at corresponding regions.

The ratio $\frac{\text{ventral horns (V)}}{\text{dorsal horns (D)}}$ increases from the first to the fourth lumbar region, and is approximately the same in the fourth and fifth.

From the fifth lumbar region downwards to the end of the cord the ratio decreases, being as 1 : 1 in the first coccygeal region.

The ratio increases very rapidly in both the third and fourth lumbar regions. It decreases very rapidly in the sixth and seventh lumbar and first sacral regions, and more especially in the seventh lumbar.

The ratio $\frac{\text{ventral horns (V)}}{\text{dorsal horns (D)}}$ being approximately the same in the second sacral and first lumbar regions, the decrease between the fifth lumbar and second sacral region is seen to be less rapid than the corresponding increase between the first and fifth lumbar regions.

B4. *Ratio of the Dorsal and of the Ventral Horns of the Grey Substance to the Total Area of the Cross-section of the Cord.*

Since the percentage of grey substance to the cross-section of the cord and the ratio $\frac{\text{ventral horns}}{\text{dorsal horns}}$ have already been represented graphically in Charts III (G) and XV, no curves accompany the following table (XVI) giving the ratios $\frac{\text{dorsal horns}}{\text{total area of cross-section}}$, $\frac{\text{ventral horns}}{\text{total area of cross-section}}$.

Table XVI.—Ratio of the Dorsal and of the Ventral Horns of the Grey Substance to the Area of the Cross-section of the Cord taken as Unity.

Region of cord.		Distance in cm. from uppermost section of I Lumbar region.	Dorsal horns / Area of cross-section of cord.	Mean.	Ventral horns / Area of cross-section of cord.	Mean.
Dorsal (thoracic)—						
VI		—	0·0584	—	0·0692	
XII		—	0·0690	—	0·0960	
Lumbar—						
I	Upper	0·142	0·0860	0·0871	0·1146	0·1171
	Middle	0·268	0·0845		0·1118	
	Lower	0·608	0·0907		0·1249	
II	Upper	0·846	0·1029	0·1016	0·1431	0·1491
	Middle	1·058	0·1039		0·1522	
	Lower	1·302	0·0980		0·1522	
III	Upper	1·558	0·0934	0·0908	0·1821	0·1913
	Middle	1·681	0·0922		0·1911	
	Lower	1·918	0·0869		0·2007	
IV	Upper	2·064	0·0826	0·0864	0·2388	0·2601
	Middle	2·244	0·0905		0·2686	
	Lower	2·484	0·0861		0·2728	
V	Upper	2·608	0·0874	0·0984	0·2792	0·2975
	Middle	2·794	0·0992		0·3005	
	Lower	2·944	0·1085		0·3127	
VI	Upper	3·154	0·1202	0·1267	0·3182	0·3058
	Middle	3·205	0·1209		0·3039	
	Lower	3·328	0·1391		0·2952	
VII	Upper	3·450	0·1608	0·1692	0·2957	0·2870
	Middle	3·557	0·1770		0·2826	
	Lower	3·642	0·1700		0·2827	
Sacral—						
I	Upper	3·782	0·1839	0·1943	0·2722	0·2706
	Middle	3·906	0·2010		0·2678	
	Lower	4·057	0·1980		0·2717	
II	Upper	4·197	0·1988	0·1985	0·2707	0·2699
	Middle	4·316	0·1927		0·2714	
	Lower	4·509	0·2040		0·2677	
III	Upper	4·623	0·1948	0·2107	0·2688	0·2659
	Middle	4·723	0·2145		0·2638	
	Lower	4·858	0·2228		0·2653	
IV	Upper	4·974	0·2310	0·2336	0·2606	0·2601
	Middle	5·138	0·2373		0·2597	
	Lower	5·312	0·2325		0·2602	
Coccygeal—						
I	Upper	5·486	0·2565	0·2565	0·2695	0·2705
	Middle	5·622	0·2572		0·2720	
	Lower	5·726	0·2559		0·2700	
II	Upper	5·847	0·2658	0·2655	0·2467	0·2498
	Middle	5·939	0·2656		0·2552	
	Lower	6·058	0·2653		0·2477	
III	Upper	6·174	0·2874	0·2821	0·2216	0·2041
	Middle	6·278	0·2766		0·1992	
	Lower	6·331	0·2823		0·1914	

The relative importance of the dorsal and the ventral horns of the grey substance to the total area of the cross-section of the cord varies considerably between the first lumbar and the third coccygeal regions. This is shown in Chart XVI.

The calculations were made with the mean figures of each ratio as given in Table XVI. The ratio $\frac{\text{dorsal horns}}{\text{total area of cross-section of cord}}$ in the region of each nerve is divided by 0·0871, the value of the ratio in the first lumbar region, and similarly the ratios $\frac{\text{ventral horns}}{\text{total area of cross-section of cord}}$ are each divided by 0·1171, the value of that ratio for the first lumbar region.

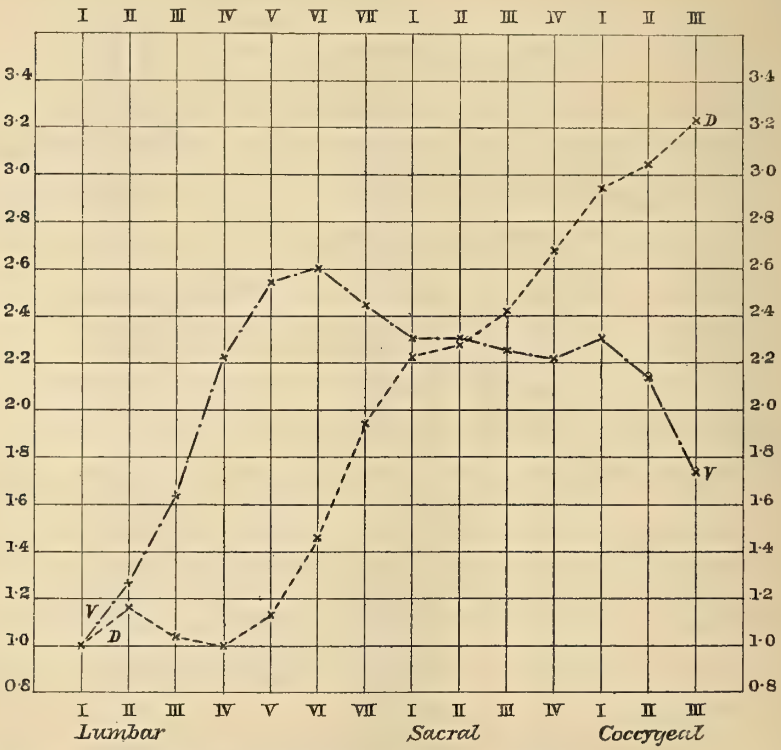


Chart XVI.—Curves showing the Relative Values of the Ratios—

$\frac{\text{Dorsal Horns}}{\text{Area of Cross-section of Cord}}$, $\frac{\text{Ventral Horns}}{\text{Area of Cross-section of Cord}}$.

(D, broken line and cross). (V, line, dot, and cross).

(Table XVII.)

The ratio in the first lumbar region (see Table XVI) is in each case taken as unity. The abscissæ denote spinal nerve regions, the ordinates relative value at corresponding regions.

It will be seen that whereas the ratio $\frac{\text{ventral horns (V)}}{\text{total area of cross-section}}$ taken as unity in the first lumbar region increases rapidly to the sixth lumbar, it decreases at the first sacral, remains constant at the second, falls slightly from this to the fourth sacral, and while rising again in the first coccygeal region, subsequently falls rapidly to the third coccygeal.

On the other hand, the value of the ratio $\frac{\text{dorsal horns (D)}}{\text{total area of cross-section}}$ taken as unity in the first lumbar region increases slightly in the second lumbar, but falls and has practically the same value in the third and fourth lumbar regions as in the first. It then increases rapidly from the fifth lumbar region to the end of the cord.

In regarding the curves together it will be observed that the two ratios V and D, bear roughly the same proportion to each other in the second sacral region that they do in the first lumbar.

A further noteworthy point is that while the dorsal and ventral horns each constitute, relatively, a much larger portion of the total area of the cross-section of the cord in the third coccygeal than in the first lumbar region, the increase is much more important in the case of the dorsal horns (D).

Table XVII.—Relative Values of the Ratios $\frac{\text{Dorsal Horns}}{\text{Area of Cross-section of Cord}}$, $\frac{\text{Ventral Horns}}{\text{Area of Cross-section of Cord}}$. The ratio in the I Lumbar region (*vide* Table XVI) is in each case taken as unity (Chart XVI).

Region of cord.	Dorsal horns.	Ventral horns.
Lumbar—		
I	1·0	1·0
II	1·17	1·27
III	1·04	1·63
IV	0·99	2·22
V	1·13	2·54
VI	1·46	2·61
VII	1·94	2·45
Sacral—		
I	2·23	2·31
II	2·28	2·30
III	2·42	2·26
IV	2·68	2·22
Coccygeal—		
I	2·95	2·31
II	3·05	2·13
III	3·24	1·74

B5. *Ratio of the Dorsal Columns and of the Ventro-lateral Columns to the Dorsal Horns.*

Table XVIII.—Ratio of the Dorsal Columns and of the Ventro-lateral Columns to the Dorsal Horns taken as Unity in each Cross-section of the Cord (Chart XVII).

Region of cord.		Distance in cm. from uppermost section of I Lumbar region.	Dorsal columns. / Dorsal horns.	Mean.	Ventro-lateral columns. / Dorsal horns.	Mean.
Dorsal (thoracic)—						
VI		—	3·88			
XII		—	3·31			
Lumbar—						
I	Upper	0·142	2·34	2·53	6·68	6·51
	Middle	0·268	2·73		6·77	
	Lower	0·608	2·53		6·08	
II	Upper	0·846	2·20	2·18	5·11	5·17
	Middle	1·058	2·12		5·03	
	Lower	1·302	2·22		5·38	
III	Upper	1·558	2·34	2·34	5·40	5·55
	Middle	1·681	2·31		5·45	
	Lower	1·918	2·37		5·79	
IV	Upper	2·064	2·40	2·13	5·79	5·42
	Middle	2·244	1·99		5·07	
	Lower	2·484	2·00		5·40	
V	Upper	2·608	1·91	1·60	5·28	4·55
	Middle	2·794	1·51		4·45	
	Lower	2·944	1·38		3·93	
VI	Upper	3·154	1·12	1·08	3·54	3·41
	Middle	3·205	1·14		3·62	
	Lower	3·328	0·383		3·07	
VII	Upper	3·450	0·825	0·776	2·53	2·36
	Middle	3·557	0·736		2·15	
	Lower	3·642	0·769		2·41	
Sacral—						
I	Upper	3·782	0·662	0·602	2·28	2·14
	Middle	3·906	0·582		2·05	
	Lower	4·057	0·564		2·09	
II	Upper	4·197	0·568	0·595	2·09	2·06
	Middle	4·316	0·623		2·12	
	Lower	4·509	0·595		1·99	
III	Upper	4·623	0·598	0·577	2·14	1·92
	Middle	4·723	0·604		1·90	
	Lower	4·858	0·595		1·75	
IV	Upper	4·974	0·500	0·498	1·68	1·65
	Middle	5·138	0·484		1·62	
	Lower	5·312	0·510		1·65	
Coccygeal—						
I	Upper	5·486	0·391	0·392	1·43	1·43
	Middle	5·622	0·411		1·41	
	Lower	5·726	0·376		1·45	
II	Upper	5·847	0·368	0·367	1·46	1·44
	Middle	5·939	0·376		1·42	
	Lower	6·058	0·358		1·43	
III	Upper	6·174	0·281	0·280	1·38	1·51
	Middle	6·278	0·272		1·61	
	Lower	6·331	0·288		1·54	

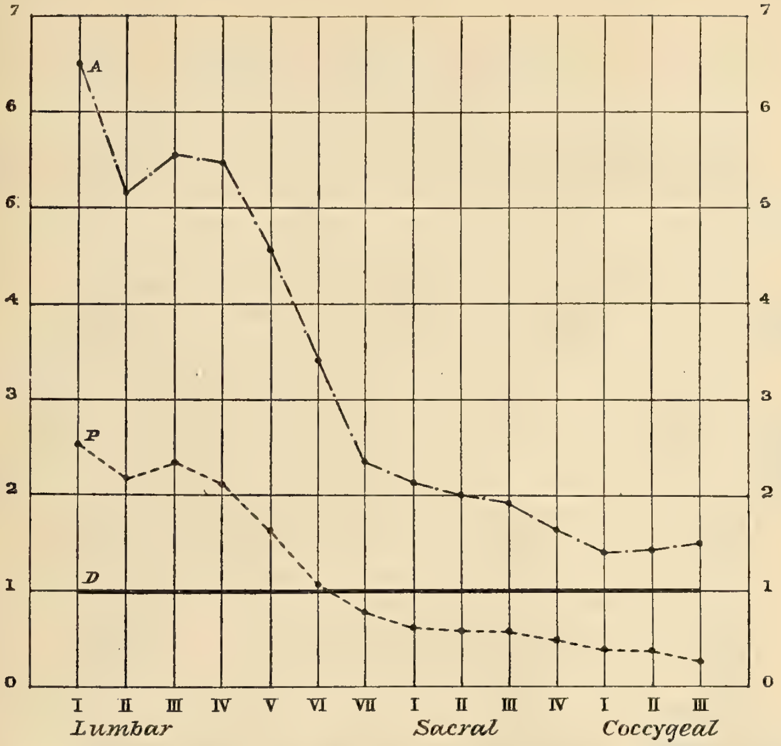


CHART XVII.—Curves showing the Ratio of the Dorsal Columns (P, broken line) and of the Ventro-lateral columns (A, line and dot) to the Dorsal Horns (D).

The dorsal horns taken as unity in each cross-section of the cord. Mean results are plotted (Table XVIII). The abscissæ denote spinal nerve regions, the ordinates ratio at the corresponding regions.

(*a*) Ratio $\frac{\text{dorsal columns (P)}}{\text{dorsal horns (D)}}$ (Chart XVII, Table XVIII).

The ratio $\frac{\text{dorsal columns (P)}}{\text{dorsal horns (D)}}$ decreases from the first lumbar region to the end of the cord.

The decrease in ratio is very rapid from the first lumbar to the first sacral region. It occurs chiefly in the fifth, sixth, and seventh lumbar regions, and more particularly in the fifth and sixth. The ratio remains very much the same from the first to the third sacral region.

(*b*) Ratio $\frac{\text{ventro-lateral columns (A)}}{\text{dorsal horns (D)}}$ (Chart XVII, Table XVIII).

The ratio $\frac{\text{ventro-lateral columns (A)}}{\text{dorsal horns (D)}}$ decreases from the first lumbar region to the first coccygeal, and increases slightly in the lower coccygeal regions.

The decrease is slight from the first to the fourth lumbar, but very rapid from the fourth to the seventh lumbar region.

The ratio never becomes as 1 : 1.

B6. *Ratio of the Dorsal Columns and of the Ventro-lateral Columns to the Ventral Horns.*

Table XIX.—Ratio of the Dorsal Columns and of the Ventro-lateral Columns to the Ventral Horns taken as Unity in each Cross-section of the Cord (Chart XVIII).

Region of cord.	Distance in cm. from uppermost section of I Lumbar region.	Dorsal columns. / Ventral horns.	Mean.	Ventro-lateral columns. / Ventral horns.	Mean.
Dorsal (thoracic)—					
VI	—	—	—	9 ·27	
XII	—	—	—	6 ·31	
Lumbar—					
I Upper	0 ·142	1 ·93	1 ·93	5 ·01	4 ·85
Middle	0 ·268	2 ·05		5 ·12	
Lower	0 ·608	1 ·84		4 ·42	
II Upper	0 ·846	1 ·58	1 ·48	3 ·67	3 ·52
Middle	1 ·058	1 ·45		3 ·43	
Lower	1 ·302	1 ·43		3 ·47	
III Upper	1 ·558	1 ·20	1 ·11	2 ·77	2 ·63
Middle	1 ·681	1 ·12		2 ·63	
Lower	1 ·918	1 ·03		2 ·51	
IV Upper	2 ·064	0 ·829	0 ·709	2 ·00	1 ·81
Middle	2 ·244	0 ·668		1 ·71	
Lower	2 ·484	0 ·632		1 ·76	
V Upper	2 ·608	0 ·599	0 ·571	1 ·65	1 ·50
Middle	2 ·794	0 ·512		1 ·47	
Lower	2 ·944	0 ·604		1 ·37	
VI Upper	3 ·154	0 ·422	0 ·444	1 ·34	1 ·41
Middle	3 ·205	0 ·453		1 ·44	
Lower	3 ·328	0 ·458		1 ·45	
VII Upper	3 ·450	0 ·448	0 ·457	1 ·37	1 ·42
Middle	3 ·557	0 ·463		1 ·43	
Lower	3 ·642	0 ·461		1 ·46	
Sacral—					
I Upper	3 ·782	0 ·447	0 ·431	1 ·54	1 ·53
Middle	3 ·906	0 ·437		1 ·54	
Lower	4 ·057	0 ·411		1 ·52	
II Upper	4 ·197	0 ·417	0 ·437	1 ·53	1 ·52
Middle	4 ·316	0 ·442		1 ·52	
Lower	4 ·509	0 ·453		1 ·52	
III Upper	4 ·623	0 ·434	0 ·449	1 ·55	1 ·50
Middle	4 ·723	0 ·468		1 ·47	
Lower	4 ·858	0 ·445		1 ·47	
IV Upper	4 ·974	0 ·443	0 ·447	1 ·49	1 ·48
Middle	5 ·138	0 ·442		1 ·48	
Lower	5 ·312	0 ·456		1 ·48	
Coccygeal—					
I Upper	5 ·486	0 ·372	0 ·373	1 ·36	1 ·35
Middle	5 ·622	0 ·392		1 ·33	
Lower	5 ·726	0 ·356		1 ·37	
II Upper	5 ·847	0 ·397	0 ·390	1 ·56	1 ·53
Middle	5 ·939	0 ·392		1 ·48	
Lower	6 ·058	0 ·383		1 ·55	
III Upper	6 ·174	0 ·362	0 ·388	1 ·78	2 ·10
Middle	6 ·278	0 ·378		2 ·23	
Lower	6 ·331	0 ·425		2 ·28	

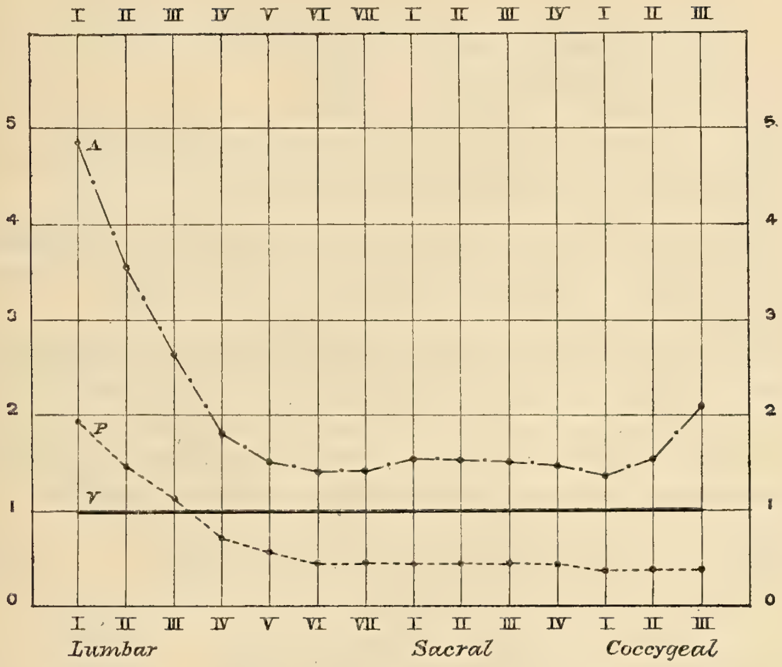


Chart XVIII.—Curves showing the Ratio of the Dorsal Columns (P, broken line) and of the Ventro-lateral Columns (A, line and dot) to the Ventral Horns (V).

The ventral horns taken as unity in each cross-section of the cord. Mean results are plotted (Table XIX). The abscissæ denote spinal nerve regions, the ordinates ratio at the corresponding regions.

(*a*) Ratio $\frac{\text{dorsal columns (P)}}{\text{ventral horns (V)}}$ (Chart XVIII, Table XIX).

The ratio $\frac{\text{dorsal columns (P)}}{\text{ventral horns (V)}}$ decreases from the first to the sixth lumbar region, the decrease being rapid to the fourth. It remains nearly the same from the sixth lumbar to the fourth sacral region, decreases slightly in the first coccygeal region and varies very little in the second and third.

(*b*) Ratio $\frac{\text{ventro-lateral columns (A)}}{\text{ventral horns (V)}}$ (Chart XVIII, Table XIX).

The ratio $\frac{\text{ventro-lateral columns (A)}}{\text{ventral horns (V)}}$ decreases rapidly from the first to the sixth lumbar region. The decrease is very marked in the second, third, and fourth, but it is less in the third than in the second, and is again less in the fourth than in the third. The ratio increases very slightly from the sixth lumbar to the first sacral region and remains approximately the same throughout the regions of the sacral nerves. A slight decrease in the first coccygeal is followed by an increase in the second and third coccygeal regions, the increase being marked in the third.

B7. *Relative Increase and Decrease in the Section Area of the Cord and of each of its Component Parts.*

A general idea of the relative increase and decrease in the section area of the cord and of its component parts is to be obtained from the following table (XX) and Chart XIX.

The section area of each constituent of the cord at the particular region in which it reaches a maximum value is taken as 100. For the cord as a whole, and for the white substance and its component parts—the dorsal and the ventro-lateral columns—this region is the fourth lumbar; for the grey substance and its component parts—the dorsal and the ventral horns—the fifth lumbar.

The mean figures obtained from the three sections taken in each region were employed (Table XX) in drawing the chart:—

Table XX.—The Section Area of the Cord and of each of its Component Parts at its Maximum Value is taken as 100. The Table gives the mean percentage values of the cross-section of the cord and of each of its component parts in the region of each spinal nerve (Chart XIX).

Region of cord.	Cross-section of cord.	Grey substance.	Dorsal horns.	Ventral horns.	White substance.	Dorsal columns.	Ventro-lateral columns.
Lumbar—							
I	64	34	56	26	80	80	78
II	71	46	73	37	83	87	80
III	76	55	69	51	85	88	82
IV	100	87	84	88	100	100	100
V	94	100	100	100	89	82	90
VI	73	82	92	78	64	54	67
VII	53	63	90	53	45	38	46
Sacral—							
I	39	47	76	37	32	25	35
II	29	35	58	27	24	19	26
III	22	27	45	20	18	14	19
IV	17	21	39	15	13	11	14
Coccygeal—							
I	12	17	31	11	9	7	10
II	7	9	18	6	5	4	5
III	3	4	10	2	3	1	3

Chart XIX.

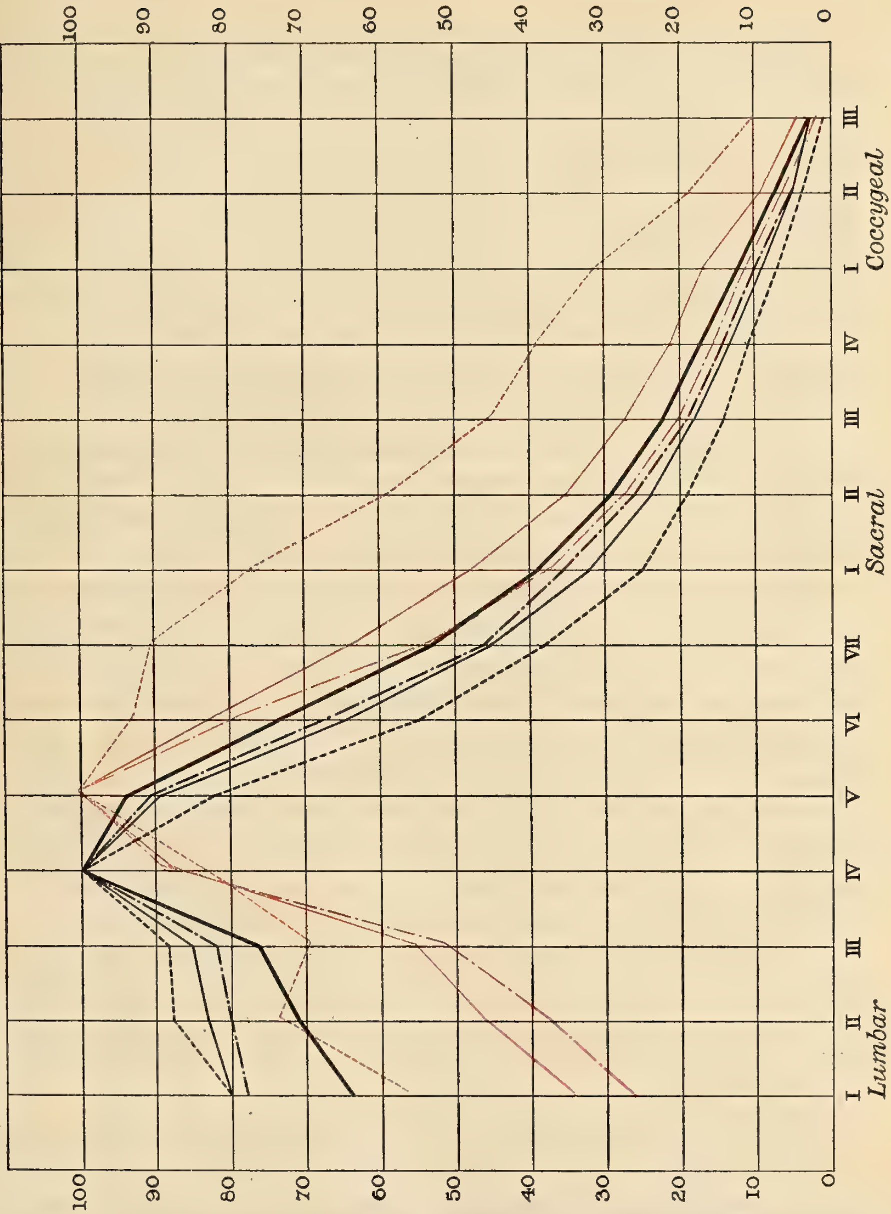


Chart XIX.—Curves showing the Relative Increase and Decrease in the Cross-sectional Area of the Cord, of the Grey and the White Substances and of their Component Parts (Table XX).

The section area at its maximum value is in each case taken as 100. The abscissæ denote spinal nerve region, the ordinates percentage value at corresponding region.

Area of cross-section of cord	Heavy black line.
Section area of grey substance	Red continuous line.
" dorsal horns	Red broken line.
" ventral horns	Red line and dot.
" white substance	Thin black continuous line.
" dorsal white columns	Thin black broken line.
" ventro-lateral white columns	Thin black line and dot.

Thus it is seen that:—

(1) In sectional area, both the white substance as a whole, and the ventro-lateral and dorsal columns, increase with the cord from the first to the fourth lumbar region, and decrease together with the cord from the fourth lumbar to the third coccygeal region.

(2) The increase in the white substance is relatively less than that in the cord, whereas the decrease is relatively greater.

(3) Of the white substance, both the ventro-lateral and dorsal columns increase relatively less than the cord, and decrease relatively more to the second coccygeal region. From this region the decrease in the ventro-lateral columns is relatively less than that in the cord, though the decrease in the dorsal columns continues to be relatively greater.

(4) The ventro-lateral columns increase relatively rather more than the dorsal columns, and decrease relatively less.

(5) The decrease in the dorsal columns is relatively greater than the decrease in any other component part of the cord.

(6) In sectional area, both the grey substance as a whole, and the ventral and dorsal horns, increase with the cord and white substance, from the first to the fourth lumbar region, and *continue to increase to the fifth lumbar region*, whilst the cord and white substance are decreasing. The grey substance and the ventral and dorsal horns decrease with the cord and white substance from the fifth lumbar to the third coccygeal region.

(7) The increase of the grey substance is relatively much greater than the increase of the cord, and is still greater than the increase in the white substance. The decrease is relatively less than that of the cord, and much less than that of the white substance and its component parts.

(8) Of the grey substance, both the ventral and dorsal horns increase relatively more than the cord, the white substance and its component parts.

(9) The increase in the "ventral horns" is relatively much greater than that in any other component part of the cord, and is considerably greater than the increase in the "dorsal" horns.

(10) The ventral horns decrease in much the same proportion as the cord (or relatively rather less to the seventh lumbar region and subsequently rather more). They decrease relatively less than the white substance and its component parts, to the second coccygeal region, where the decrease becomes relatively greater than the decrease in the ventro-lateral columns.

(11) The decrease of the "dorsal horns" is relatively less than that of the cord and of any other part of the same.

The rate of increase or decrease of the section area of the cord, of the grey and the white substances and of their component parts, between the region of one spinal nerve and that of the next, is shown in the following chart (XX). The calculations were made with the mean sectional area figures as given in Tables I to V, and XII, XIII.

Taking the total area of the cross-sections of the cord as the example (Chart XX, heavy black line, S), the method employed was as follows: The mean sectional area of the first lumbar region was taken as unity. The value of the second point of the curve was obtained by dividing the mean sectional area of the second lumbar region by that of the first lumbar; similarly the value of the third point of the curve was obtained by dividing the mean sectional area of the third lumbar region by the mean sectional area of the second lumbar, and so on throughout the remaining regions of the cord.

To render comparison easy, the curves are arranged in three separate groups in the same chart. The rate of increase and decrease in the section area of the cord, the grey and the white substance is indicated by the curves in Group S, that of the white substance and its component parts, the ventro-lateral and the dorsal columns, in Group Z, and that of the grey substance and the ventral and dorsal horns in Group B.

All the curves are comparable one with another.

Table XXI.—Rate of Increase and Decrease in the Sectional Area of the Cord and of each of its Component Parts. The mean sectional area of the first lumbar region is in each case taken as unity (Chart XX).

Region of cord.	Cross-section of the cord.	Grey substance.	Dorsal horns.	Ventral horns.	White substance.	Dorsal columns.	Ventro-lateral columns.
Lumbar—							
I	1·0	1·0	1·0	1·0	1·0	1·0	1·0
II	1·1	1·4	1·3	1·4	1·1	1·1	1·0
III	1·1	1·2	1·0	1·4	1·0	1·0	1·0
IV	1·3	1·6	1·2	1·7	1·2	1·1	1·2
V	0·9	1·1	1·1	1·1	0·9	0·8	0·9
VI	0·8	0·8	1·0	0·8	0·7	0·7	0·7
VII	0·7	0·8	1·0	0·7	0·7	0·7	0·7
Sacral—							
I	0·7	0·7	0·8	0·7	0·7	0·6	0·7
II	0·8	0·8	0·8	0·8	0·7	0·8	0·7
III	0·7	0·8	0·8	0·7	0·7	0·8	0·7
IV	0·8	0·8	0·9	0·8	0·7	0·8	0·7
Coccygeal—							
I	0·7	0·8	0·8	0·7	0·7	0·6	0·7
II	0·5	0·5	0·6	0·5	0·6	0·5	0·6
III	0·5	0·5	0·5	0·4	0·5	0·4	0·6

Thus the total area of the cord in the second lumbar is 1·1 times its area in the first, from thence to the third lumbar the increase is at the same rate

Chart XX.

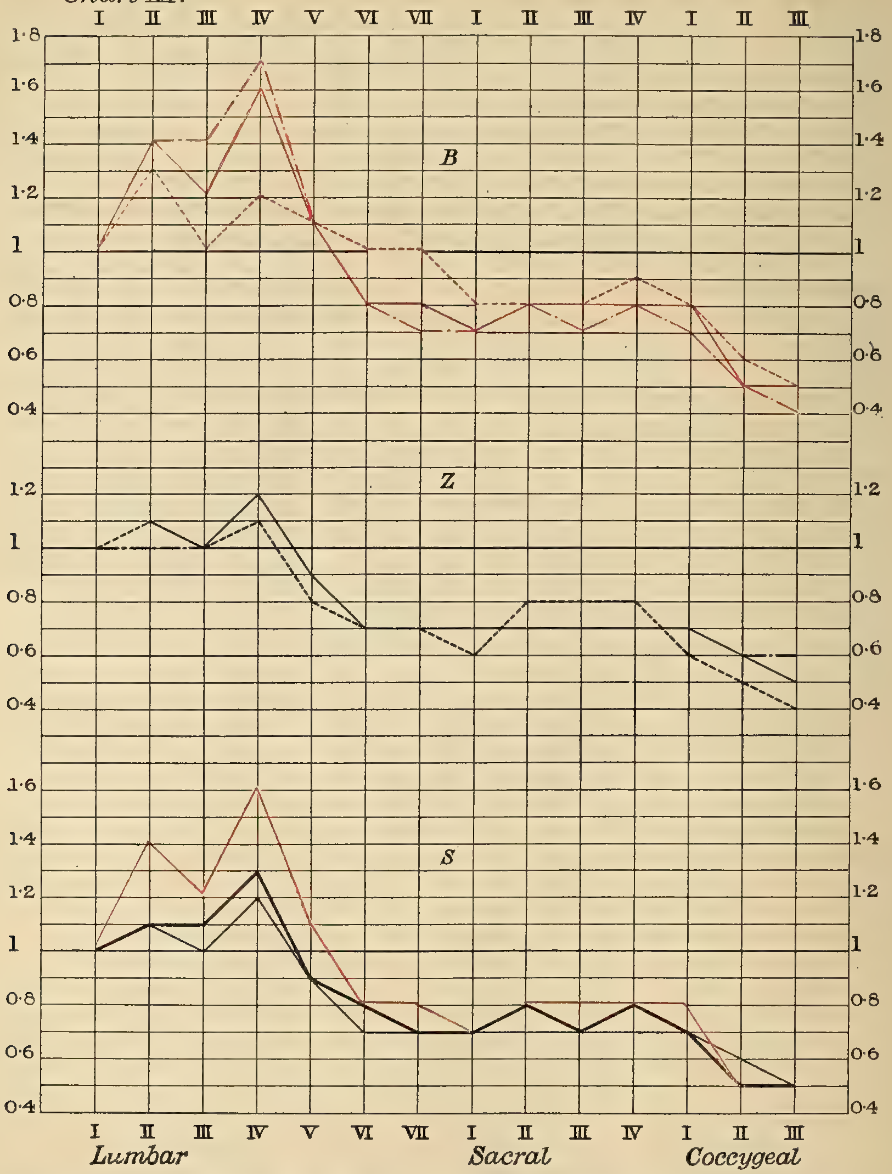


CHART XX.—Curves showing the Rate of Increase or Decrease of the Section Areas of the Cord, the Grey and the White Substances, and their Component Parts (Table XXI).

The mean sectional area of the first lumbar region is in each case taken as unity. The abscissæ denote spinal nerve regions, the ordinates rate of increase or decrease.

Group S.—Cross-section of cord	Heavy black line.
Total grey substance	Red line.
Total white substance	Thin black line.
Group Z.—Total white substance	Black continuous line.
Dorsal white columns	Black interrupted line.
Ventro-lateral white columns	Black line and dot.
Group B.—Total grey substance	Red continuous line.
Dorsal horns	Red interrupted line.
Ventral horns	Red line and dot.

the area at the third lumbar being 1·1 times the area at the second. At the fourth lumbar the increase is more rapid, namely, 1·3, but at the fifth lumbar the section area is only 0·9 that at the fourth lumbar, and so on.

Summary.

From the examination of the cross-sections of the lumbo-sacral-coccygeal cord of the Macaque monkey (*Macacus sinicus*), it is seen that:—

(1) The maximum section area of the cord, of the white substance as a whole, as well as of the dorsal and the ventro-lateral columns is found in the fourth lumbar region.

(2) The maximum section area of the grey substance as a whole, and of the dorsal and the ventral horns, is found in the fifth lumbar region.

(3) Reckoning the cross-sectional area of the cord as 100, the maximum percentage of the white substance as a whole, and of the dorsal and the ventro-lateral columns, is found in the first lumbar region.

(4) The maximum percentage of the grey substance is reached in the first coccygeal region.

(5) Reckoning the total area of the grey substance in each cross-section of the cord as 100, the maximum percentage of the dorsal horns is found in the third coccygeal region, and that of the ventral horns in the fifth lumbar region.

For the furtherance of this research on the spinal cord of the Macaque monkey, a series of drawings has been made of the minute structure of the grey substance, for the purpose of determining the arrangement of the cell groups at the different levels of the cord.

It has been thought advisable to deal with this portion of the research in a separate paper.

In conclusion, I desire to thank Professor Gotch for having allowed me to carry out this research in the Physiological Laboratory, Oxford, and for his kind advice. I also wish to express my sincere thanks to many others who have assisted and encouraged me, especially to Dr. G. Mann, at whose suggestion the work was undertaken, for his guidance and kind help; to Dr. G. J. Burch, for his ready assistance in many ways, and to Professor C. S. Sherrington (Liverpool), for his valuable criticism and suggestions.

Chart XXI.

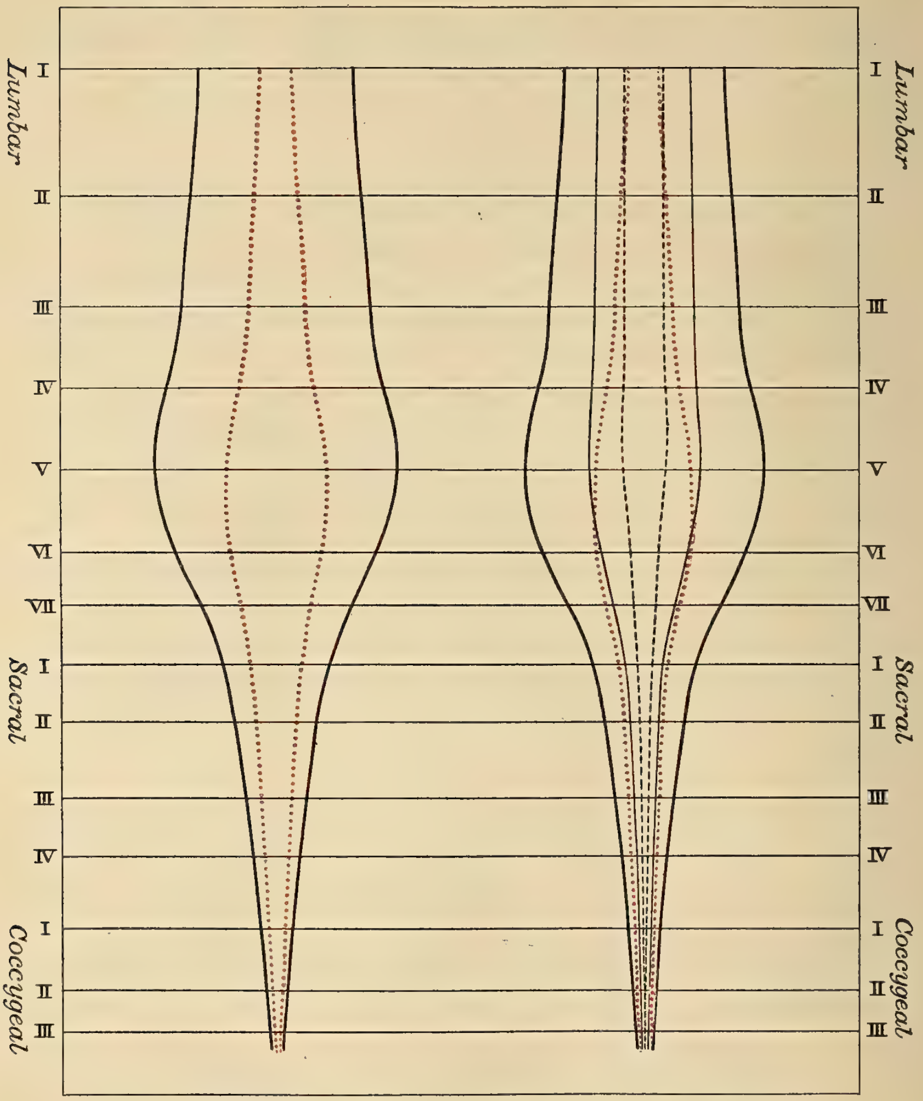


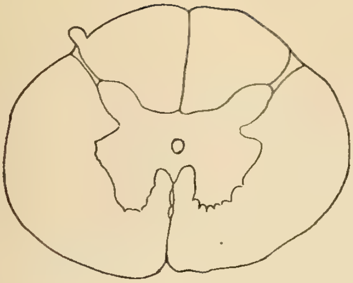
Fig. 1. Fig. 2.

CHART XXI.

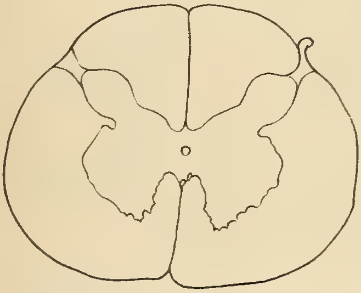
C1. DIAGRAM.—General Configuration of the Lumbo-sacral-coccygeal Cord in Sectional Area.

The figures (1, 2) are drawn to scale. The actual length of the cord is increased two-and-a-half times, and the sectional area to twice its actual value. Mean sectional area figures are employed. It must be remembered that the *sectional area* is plotted in one dimension, and that the *width* of the cord from side to side is not represented* (see p. 144).

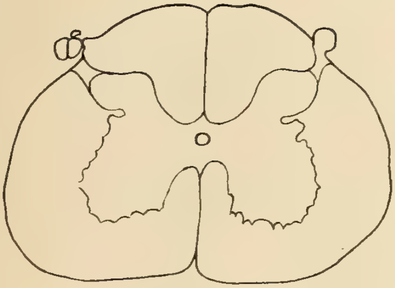
Fig. 1.—Area of the cross-section of the cord	Heavy black line.
Section area of grey substance	Red dotted line.
" 2.—Area of the cross-section of the cord	Heavy black line.
Section area of the grey substance.....................	Red dotted line.
" " dorsal white columns	Thin black broken line.
" " ventro-lateral white columns...	Thin black continuous line.



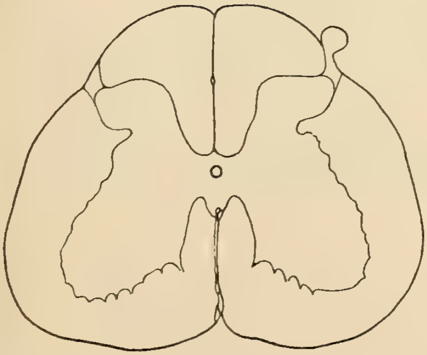
I L.



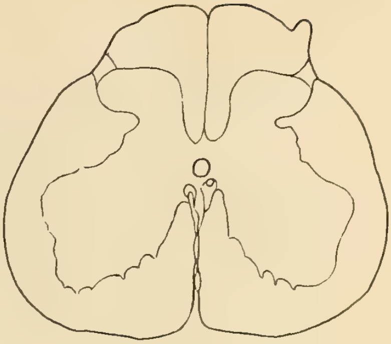
II L.



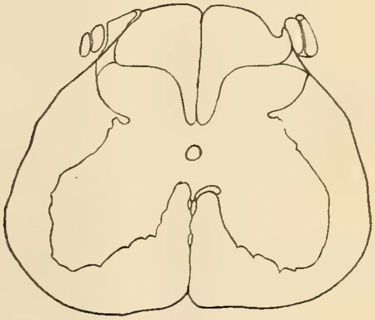
III L.



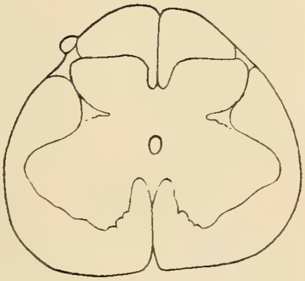
IV L.



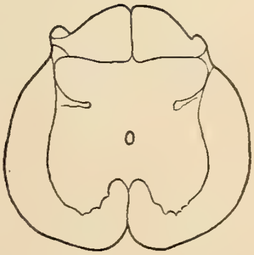
V L.



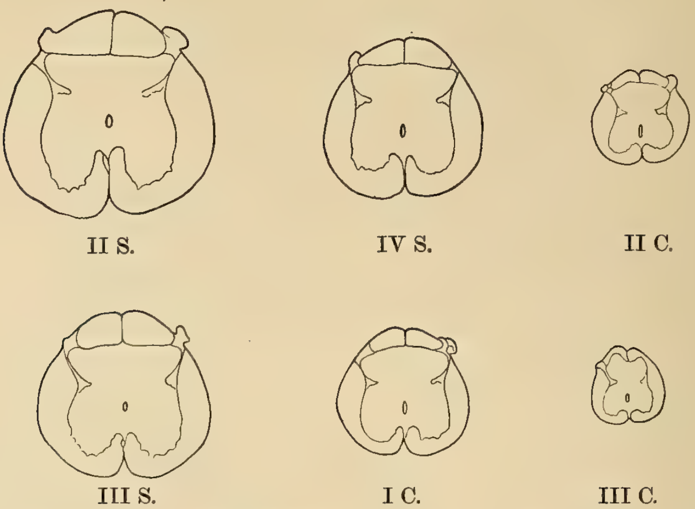
VI L.



VII L.



I S.



C2.—Outline Drawings of the Cross-section of the Cord in the Region of each Spinal Nerve. Magnification, 10 diameters.

	Distance in cm. from uppermost section of I lumbar region.		Distance in cm. from uppermost section of I lumbar region.
I Lumbar (upper)	0·142	I Sacral (upper)	3·792
II Lumbar (middle)	1·068	II Sacral (middle)	4·293
III Lumbar (middle)	1·710	III Sacral (middle)	4·768
IV Lumbar (lower)	2·338	IV Sacral (upper)	5·054
V Lumbar (lower)	2·998	I Coccygeal (middle) ...	5·644
VI Lumbar (middle)	3·191	II Coccygeal (middle) ...	5·976
VII Lumbar (upper)	3·518	III Coccygeal (upper)	6·193

Footnote to p. 142.

* The subject of cubic volume has not been entered into, but a passing reference is made to the relatively small volume of grey substance in the sixth lumbar region of the present monkey. In a region of such great physiological importance this is a matter of surprise, and may be due to error; but against this view is the fact that, on examining the cord of another Macaque monkey, the volume of the grey substance in the fifth lumbar was again found to be greater than that of the sixth or seventh, though the difference between the fifth and sixth was not so marked as in the present instance. A further examination of several Macaque cords would be necessary to determine this point.

Cyanogenesis in Plants.

Part IV.—*The Occurrence of Phaseolunatin in Common Flax* (Linum usitatissimum).

By Wyndham R. Dunstan, M.A., LL.D., F.R.S., T. A. Henry, D.Sc., Principal Assistant in the Scientific and Technical Department of the Imperial Institute, and S. J. M. Auld, Ph.D.

(Received April 11,—Read May 10, 1906.)

In Part III of this series of papers* it was shown that the seeds of *Phaseolus lunatus*, as produced by the uncultivated plant in Mauritius, contained a new cyanogenetic glucoside, phaseolunatin, which was proved to have the constitution of a dextrose ether of acetonecyanhydrin. Phaseolunatin was further shown to undergo hydrolysis by mineral acids or by the action of the characteristic, emulsin-like enzyme also present in the seeds, yielding, as final products, acetone, hydrocyanic acid, and dextrose.

This decomposition of the glucoside by the enzyme takes place when the ground seeds of *Phaseolus lunatus* are mixed with water, and from such a preparation it is comparatively easy to isolate and identify the hydrolytic products, acetone and hydrocyanic acid. The simultaneous production, therefore, of these two substances from plants by mere contact with water may be taken as indicating the occurrence in such plants of phaseolunatin or some similar derivative of acetonecyanhydrin, and of an enzyme capable of decomposing this compound under the conditions specified.

Before the isolation of phaseolunatin, it was observed by Van Romburgh† that various plants, on crushing with water and subsequent distillation, yielded acetone, and that in some cases this was accompanied by hydrocyanic acid. The plants mentioned by Van Romburgh as yielding both these products are *Phaseolus lunatus*, *Manihot utilissima* (the Cassava plant from which the tapioca of commerce is prepared), *Manihot glaziovii* (the Ceara rubber tree), and *Hevea brasiliensis* (the Para rubber tree). This author stated that he was at first inclined to associate the simultaneous production of acetone and hydrocyanic acid from these plants with the occurrence in them of a compound of these two substances, but his subsequent discovery that several plants, notably *Erythroxylon Coca*, from which the "Coca leaves," largely used as a stimulant in South America, are procured, and *Pogostemon cristatus*, yielded acetone unaccompanied by hydrocyanic acid, led him to abandon this idea, and he suggested that the acetone might occur in the form of a glucoside.

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* 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† 'Annales du Jardin Botanique de Buitenzorg,' 1899, vol. 2, 1, p. 2.

Quite recently Van Itallie has observed* that the leaves of *Thalictrum aquilegifolium* yield acetone and hydrocyanic acid under similar conditions, and he has suggested that this plant may contain the glucoside phaseolunatin which we had isolated previously from the seeds of *Phaseolus lunatus.*

A systematic investigation of these various plants is being made, as a part of the general work on cyanogenesis we have undertaken, with a view to ascertaining definitely whether they contain phaseolunatin, and in the present and succeeding papers two cases are dealt with, namely, the "flax" and "cassava" plants. The seeds of the Para rubber tree are at present under investigation, and the examination of the other plants mentioned will be commenced as soon as material, which is rather difficult to procure, becomes available.

Cyanogenesis in flax was first observed by Jorissen,† who stated (1) that, when linseed meal (ground flax seed) is allowed to stand with warm water at 25° C., hydrocyanic acid is produced, and may be obtained by distillation of the mixture, and (2) that the acid does not exist preformed in the seed, since the latter does not yield it when placed in boiling water. This author suggested that flax seed probably contains a substance on which emulsin acts in the same way as on amygdalin, and further noted that linseed meal has the property of decomposing amygdalin, liberating benzaldehyde, hydrocyanic acid, and dextrose. In subsequent papers, Jorissen stated that both *Linum usitatissimum* and *Linum perenne* contain amygdalin in the leaves and stems, and that, in the case of the former, a notable increase in the amount of hydrocyanic acid obtainable takes place on germination of the seed. Thus, whereas a portion of one sample of flax seed yielded 0·01 per cent. of the acid, another portion of the same sample, after germination, yielded 0·07 per cent.‡

Subsequently, Jorissen and Hairs§ succeeded in isolating the cyanogenetic glucoside of flax in a crystalline condition. They named it "linamarin," and described it as crystallising in groups of colourless needles, melting at 134° C., having a cool and bitter taste, and being readily soluble in water.

They assigned no formula to linamarin, but stated that, on combustion, it gave the following results: carbon, 47·88 per cent.; hydrogen, 6·68 per cent.; oxygen, 39·89 per cent.; and nitrogen, 5·55 per cent.; and that, on hydrolysis, by heating with dilute mineral acids, or by the addition of linseed meal to an aqueous solution of the glucoside, the latter was decomposed, yielding hydrocyanic acid, a reducing sugar, and a volatile ketone, which gave the iodoform reaction. Jorissen and Hairs also examined the enzyme contained in embryonic flax plants, and observed that, whilst it had the property of hydrolysing both linamarin and amygdalin, the emulsin of almonds was incapable of decomposing linamarin.

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* 'Journ. Pharm. Chim.,' 1905, vol. 6, pp. 22, 337.

† 'Bull. Acad. Roy. Belg.,' 1883 (iii), vol. 5, p. 750.

‡ *Loc. cit.*, 1884 (iii), vol. 6, p. 718, and vol. 7, p. 736.

§ *Loc. cit.*, 1891 (iii), vol. 21, p. 529.

Van de Ven, who attempted to repeat the work of Jorissen and Hairs, using flax seed as a source of the glucoside, did not succeed in isolating linamarin, and he was also unable to find that hydrocyanic acid could be obtained from the seed.* Jouck, however,† was able to confirm and extend Jorissen and Hairs' observations, and identified the volatile ketone produced by the hydrolysis of the flax glucoside as acetone. This author, however, found, in opposition to Jorissen and Hairs, that linamarin is decomposed by the emulsin of almonds.

Preliminary Experiments.

In the present investigation, attempts were first made to utilise flax seed cake (linseed cake) as a raw material for the isolation of the glucoside, but it was found that, although distinct evidence of the presence of a cyanogenetic glucoside in the cake was readily obtainable, the quantity present was so small that it was impossible to isolate it in a crystalline condition. It was considered advisable, therefore, to have recourse to the use of young flax plants as a source of linamarin. The young flax plants for the investigation were grown during the summer of 1905, partly at the Imperial Institute and partly by permission of Professor J. B. Farmer, F.R.S.—to whom we are indebted for the help thus rendered—at the Chelsea Physic Garden. Small supplies of immature flax were also received during the earlier part of the investigation from Mr. F. Barbour, of the Department of Agriculture and Technical Instruction in Ireland, to whom our thanks are also due.

As a preliminary measure, the amount of glucoside present in whole flax plants, including roots, at various stages of growth was determined, with a view to the selection of the richest material. This estimation was carried out by completely extracting a weighed quantity of the air-dried plant with alcohol, distilling the solvent from the extract, dissolving the residue in water, hydrolysing the glucoside contained in this by boiling with hydrochloric acid, and finally distilling off and estimating the hydrocyanic acid in the distillate. Detailed accounts of the methods of carrying out the estimation of the amounts of cyanogenetic glucoside contained in plants are given in the preceding papers of this series.*

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* Van Rijn, 'Die Glykoside,' 1900.

† 'Beitrag zur Kenntnis der Blausäure abspaltenden Glycoside,' Inaug. Diss., Strassburg, 1902.

The results obtained by the estimation of the amounts of hydrocyanic acid yielded under these conditions by young flax plants were as follows:—

Height of flax plants in inches.	Grown at—	Hydrocyanic acid found.	Glucoside calculated.
		per cent.	per cent.
Seed	—	0·008	0·07
1— 1·5	Imperial Institute	0·15	1·4
2— 3	" "	0·17	1·5
3— 4	" "	0·15	1·4
4— 5	Physic Garden, Chelsea	0·13	1·2
5— 6	" "	0·10	0·9
6— 7	" "	0·10	0·9
8—10	" "	0·08	0·7
12—15	" "	0·07	0·6
15—18	" "	0·03	0·3
18	" "	0·009	0·08
18	" "	None	None

These results are of special interest since, apart from indicating the stages in the growth of the plant at which the isolation of the glucoside could be most hopefully undertaken, they show that the course of cyanogenesis in the flax plant is different from that in *Lotus arabicus* and in *Sorghum vulgare.* In each of these two plants it has been shown in the first two papers of this series† that the amount of the characteristic glucoside present steadily increases until the plants approach maturity, then decreases, and that none is present in the seed. In flax, on the other hand, the seed contains a small amount of the glucoside which increases on germination, reaches a maximum at a very early stage in the growth of the plant (when it is from 2 to 3 inches high) and then diminishes steadily and finally disappears altogether. These results on the whole confirm those of Jorissen, who also observed that a very large increase of the glucoside occurred with the germination of the seed, though the amounts recorded by him are smaller than those now found. These differences are probably due to the fact that Jorissen estimated the amount of glucoside present by determining the amount of hydrocyanic acid formed, by merely moistening a known weight of ground seed. Under these conditions it is improbable that the whole of the glucoside undergoes hydrolysis, and for this reason Jorissen's results are probably too low.

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* Dunstan and Henry, 'Phil. Trans.,' B, 1901, vol. 194, p. 515; A, 1902, vol. 199, p. 399; and 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† *Loc. cit.*

Isolation of the Glucoside.

Flax plants (stems, leaves and roots) of about 4 or 5 inches in height were thoroughly dried at a temperature not exceeding 40° C. The dried material was ground to a fine powder and completely extracted with 90 per cent. alcohol. The greater part of the solvent was then distilled off and the residue poured into water, whereby chlorophyll, oily and resinous matters, were precipitated. The aqueous liquid after decantation was decolorised by the addition of a slight excess of lead acetate, and the filtrate, after treatment with sulphuretted hydrogen, to remove the excess of lead, was evaporated down to a low bulk under reduced pressure and set aside for a time. This purified and decolorised extract was very rich in the cyanogenetic glucoside, but it could not be induced to crystallise out of the syrupy mass. The latter was, therefore, redissolved in alcohol and the solution poured into six times its volume of ether. The sticky precipitate which separated consisted principally of a mixture of dextrose and potassium nitrate. The solvent was distilled from the decanted liquid and the dry residue again dissolved in alcohol and the precipitation by means of excess of ether repeated. In this case the precipitate was principally dextrose with a small quantity of the glucoside. The solvent was again distilled from the decanted liquid and the light brown syrupy residue set aside. After some days, masses of needles arranged in rosettes began to separate and in a short time the whole of the syrup had solidified, forming a crystalline mass. This was spread on a recently-ignited porous tile and the fairly clean crystals, thus separated from the viscous mother liquor, dissolved in alcohol and recrystallised until they were colourless and of constant melting point. This material was carefully compared with phaseolunatin prepared from the seeds of *Phaseolus lunatus* and the two substances were proved to be identical.

Determinations of the melting points of phaseolunatin and the flax glucoside under the same conditions gave 138° C. (corr.) as the melting point of each, and a mixture of the two substances also melted at this temperature.

The flax glucoside was found to have the specific rotation $[\alpha]_D - 27^\circ{\cdot}4$ in alcohol at 15°; that previously recorded for phaseolunatin is $- 26^\circ{\cdot}2$ under the same conditions.

Finally both substances crystallise in the same characteristic, spreading rosettes of slender needles, and possess the same peculiar cool and bitter taste. The total quantity of the glucoside obtained by us from flax did not amount to more than 0·3 gramme, and as the results of the comparison of its physical constants with those of phaseolunatin so positively established the identity of the two substances, we considered it inadvisable to use the considerable proportion of the whole of our material, which would have been necessary, in making a combustion. The average of the combustion results quoted by Jorissen and Hairs for the flax glucoside agrees closely with those required by the formula assigned in the preceding paper of this series to phaseolunatin, viz.:—

Found by Jorissen and Hairs for linamarin—

C = 47·88 per cent.
H = 6·68 „
O = 39·89 „
N = 5·55 „

Required for phaseolunatin ($C_{10}H_{17}O_6N$)—

C = 48·1 per cent.
H = 6·8 „
O = 39·5 „
N = 5·6 „

Hydrolytic Products of the Flax Glucoside.

It has already been pointed out that Jorissen and Hairs observed that the flax glucoside was decomposed by boiling with dilute acids, liberating a reducing sugar, hydrocyanic acid and a volatile ketone. The latter was subsequently identified by Jouck as acetone. For the examination of the volatile hydrolytic products of the flax glucoside we have used a portion of the purified extract from which the glucoside was eventually induced to crystallise. The purified extract was dissolved in water, and a few cubic centimetres of 10-per-cent. hydrochloric acid added; this liquid was then distilled almost to dryness. The distillate had a strong odour of hydrocyanic acid and gave the Prussian blue reaction copiously. The remainder of the distillate was then rendered alkaline and redistilled, the first few cubic centimetres being collected separately. To this were added a few drops of benzaldehyde and a small quantity of an aqueous solution of potassium hydroxide. On standing, this mixture deposited crystals which on recrystallisation melted at 112° C., and proved to be identical with dibenzylideneacetone (melting point, 112° C.). The second volatile hydrolytic product is therefore acetone. The identity of the volatile hydrolytic products of the flax glucoside with those of phaseolunatin affords a further proof that the cyanogenetic glucoside of flax is phaseolunatin.

There seems to be no reason therefore why the name linamarin applied by Jorissen and Hairs to the cyanogenetic glucoside of flax should be retained.

Other Constituents of Flax.

It has already been mentioned that a considerable amount of potassium nitrate was found to have accumulated in the purified extract from which the flax glucoside eventually crystallised.

This occurrence of potassium nitrate with the cyanogenetic glucoside in flax is of some significance, since Treub* has pointed out that the accumulation of potassium nitrate in the stems and petioles of *Phaseolus lunatus* has an intimate connection with the secretion of phaseolunatin by that plant, and has suggested that this store of nitrate may be the raw material from which the cyanogenetic glucoside in this plant is eventually produced.

From one of the purified extracts, obtained from flax 12 inches high, grown in Ireland, a small quantity of a sugar crystallising in characteristic, cauliflower-like masses separated after the extract had stood for some time. It melted at 78° to 80° after recrystallisation from alcohol, did not reduce Fehling's solution, and was slightly dextrorotatory. When an aqueous solution was boiled with mineral acids a reducing sugar was produced. These observations seem to indicate that this material may be identical with raffinose (melting point 80° C.), but a sufficient quantity of the sugar could not be obtained for complete examination.

The Enzyme of Flax.

Preparations of this were made by macerating finely-ground flax seed (linseed) with water, previously saturated with chloroform to render the liquid antiseptic. This extract was found to have a range of activities similar to that of the emulsin of almonds, and it readily hydrolysed amygdalin and salicin. When added to an aqueous solution of the purified flax extract, prepared as already described, it speedily hydrolysed the contained phaseolunatin, yielding acetone and hydrocyanic acid, which were identified in the usual way.

Preparations of the enzyme were also found to hydrolyse phaseolunatin prepared from the seeds of wild *Phaseolus lunatus* and, *vice versâ*, preparations of the glucosidolytic enzyme contained in seeds of *Phaseolus lunatus* were found to hydrolyse the phaseolunatin obtained from flax seed, the volatile hydrolytic products being in both cases the same, viz., acetone and hydrocyanic acid.

It seems probable, therefore, that the same enzyme is contained both in *Phaseolus lunatus* seed and in flax seed. This enzyme is of the emulsin type (*i.e.*, it appears to hydrolyse β-glucosides) and exhibits similar activities, but it also presents certain well-marked differences from emulsin, which will be the subject of further investigation.

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* 'Ann. Jard. Bot. de Buitenzorg,' 1905, vol. 2, 4, p. 86.

Cyanogenesis in Plants.

Part V.—*The Occurrence of Phaseolunatin in Cassava* (Manihot Aipi *and* Manihot utilissima).

By Wyndham R. Dunstan, M.A., LL.D., F.R.S., T. A. Henry, D.Sc., Principal Assistant in the Scientific and Technical Department of the Imperial Institute, and S. J. M. Auld, Ph.D.

(Received April 11,—Read May 10, 1906.)

The "sweet" and "bitter" cassava plants are indigenous to Southern and Central America, whence they have been introduced, especially the sweet variety, into almost all tropical countries and are now widely cultivated for the sake of their edible starchy roots, which are used for the manufacture of the various forms of cassava starch, of which tapioca is perhaps the best known.

The plants are known in their native habitat by a variety of vernacular names of which only one, "manioc" or "mandioca," has come into general use. The name "cassava" seems to be restricted in South America to the flour or meal made from the roots, but outside South America this name has come to be applied to the whole plant.

There are many varieties of cassava plants in cultivation in the tropics, but these all appear to belong to either the "bitter" or "sweet" forms. These two forms were regarded by Pohl* as distinct species and were named by him *Manihot utilissima* and *Manihot Aipi* respectively.

By other botanists the "sweet" cassava is regarded as a variety or perhaps a cultivated race of *Manihot utilissima*,† whilst others take the view that Pohl's *Manihot Aipi* is identical with *Manihot palmata*.‡ Colonel Prain, Director of the Royal Gardens, Kew, whom we have consulted on this point, is of opinion that on the evidence at present available, Pohl's view, that the plants yielding the "bitter" and "sweet" cassavas are distinct species, is most likely to be the correct one.

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* 'Pl. Bras. Ic.,' i, vol. 32, p. 24.

† Compare Sagot, 'Bull. Soc. Bot. France,' 1872, vol. 18, p. 341.

‡ 'Index Kewensis,' fasc. iii, p. 162; and Peckolt, 'Pharm. Rund.,' 1886, vol. 4, p. 57.

The poisonous properties of the bitter manioc root seem to have been known to the natives of Central and Southern America from very early times and the process they use for preparing an edible meal from it seems to be designed with a view to the complete elimination of the poison. The process is described by Sagot* as follows: "The roots are scraped, peeled and washed. This clean material is then rasped and the pulp left to ferment for 24 hours. It is then placed in a long flexible basket made of plaited rushes. This is suspended by a handle at its open end and to the other a heavy weight is attached by which means the pulp is compressed and a highly poisonous juice oozes through the plaits. The pressed meal is taken out and exposed for some time over a fire and then pounded, coarsely sifted, and again exposed on a brass plate over a fire; during this operation the meal is constantly stirred so that it assumes a granular form."

The same author states that: "Sweet cassava contains so small a quantity of poisonous matter that the roots are cooked at a fire and eaten like potatoes."

Probably the earliest reference in European literature to the poison contained in cassava root is that made by Clusius,† who says: "Caçavi autem panis est quo Indi tot seculis vitam sustentarunt et hodie etiam vescuntur nostri Hispani.

"Nec minore admiratione dignum est omnem Yucam‡ in continenti nascentem tametsi quæ ad S. Dominicum nascitur (ex qua Caçavi fit) similem salutarem esse, ejusque fructum (radicem) edulem et succum inde manantem potabilem, nullamque noxam adferre; eam autem quæ ad S. Dominicum provenit (quacumque tandem ratione edatur) ejusque succum non coctum, perimere. Locorum vero naturam tanti momenti esse ut quod salubre alimentum in continenti præbet, id in omnibus insulis præsens fit venenum. Quemadmodum Columella de Persico scribit, perniciosum venenum in Perside fuisse, at ubi in Italiam translatum fuit noxium illum succum deposuisse et suavem salutaremque præbuisse."

This early observation of Clusius, that the generation of a poison in cassava is associated with the conditions under which it is grown, is of special interest in view of Wiley's statement that cassava cultivated in subtropical countries, as distinct from the tropics, becomes much less toxic.§

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* *Loc. cit.*

† 'Liber Exoticorum' (Leyden, 1605), lib. 10, fol. 339.

‡ Yuca is one of the vernacular names of the cassava plants and was in common use at that time in the Spanish South American Colonies.

§ 'Bull. U.S.A. Dept. Agric.,' Div. Chem., No. 44.

The first observation as to the volatile nature of the poison contained in the roots seems to have been made by Fermin,[*] who obtained from 50 lbs. of manioc juice 3 ounces of an intensely poisonous distillate, 35 drops of which were sufficient to poison in a few seconds a condemned slave to whom they were administered. In 1828, according to Henry and Boutron-Charlard,[†] Soubeiran and Pelletier endeavoured to isolate the toxic principle of cassava, but were unsuccessful. In 1833 Henry conducted a similar investigation, but owing to lack of material was unable to obtain any definite results. The same author, in association with Boutron-Charlard, took up the matter again in 1836,[‡] and succeeded in identifying the poisonous volatile constituent of cassava with hydrocyanic acid.

It is worth noting in this connection that although Scheele had discovered hydrocyanic acid in 1780, he was apparently unaware that it was poisonous. The toxicity of the acid was first established by Henry and Boutron-Charlard in 1833. These authors made a complete analysis of cassava, and found that the roots contained starch, free hydrocyanic acid, sugar, an organic magnesium salt, a bitter principle, a fat, a nitrogenous substance, calcium phosphate, and woody fibre. They also carried out a number of physiological experiments with fowls, and found that the distillate prepared from cassava invariably produced fatal results when administered.

The first trustworthy estimations of the amounts of hydrocyanic acid obtainable from cassava were made by Francis,[§] who established the important fact that, as regards the cassava roots grown in the West Indies, both the "sweet" and "bitter" varieties yield about the same quantities of hydrocyanic acid.

Francis' observations as to the occurrence of hydrocyanic acid in the "sweet" as well as in the "bitter" cassava grown in the West Indies were confirmed by Carmody,[||] who concluded that the principal difference between the two plants is that in the "bitter" cassava the acid is uniformly distributed throughout the root, whereas in the "sweet" cassava it is located principally in the rind of the root. Carmody also observed that in sweet cassava the acid occurs partly free and partly combined.

In 1886 Peckolt[¶] examined a number of the principal varieties of cassava grown in Brazil, and showed that most of these, both the "sweet" and

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[*] 'Mém. Acad. Sci. Berlin,' 1764.

[†] 'Mém. Acad. Royale de Médecine,' Paris, 1836, vol. 5, p. 212.

[‡] *Loc. cit.*

[§] 'Analyst,' 1870, vol. 2, p. 4.

[||] 'Lancet,' 1900,

[¶] 'Pharm. Rund.,' 1886, vol. 4, p. 227.

"bitter" forms, yielded prussic acid. This investigator made a careful search for amygdalin, but was unable to obtain evidence of the existence of this or any similar glucoside in the roots. Peckolt obtained a number of substances from cassava; these were ill-defined bodies with the exception of manihotin and manihotoxin, which were crystalline. The former is described as melting at 160°, and somewhat resembling mannitol. It does not contain nitrogen.

Peckolt also stated that hydrocyanic acid does not exist in the roots until these are withdrawn from the soil, and suggested that the acid is formed as the result of atmospheric action.

That the hydrocyanic acid does not wholly occur free in the leaves of the cassava plant was observed by van Romburgh,* who, by macerating the leaves in water, and distilling the resulting liquid, obtained a distillate containing both acetone and hydrocyanic acid, and suggested that the hydrocyanic acid occurred partly combined with acetone and partly in the form of a glucoside.

Preliminary Experiments.

The results of previous investigators on the whole tended to show either that hydrocyanic acid existed in a free state in cassava roots, or that if it were present in the form of a glucoside, the latter must be of a very unstable character, and readily decomposed with the liberation of hydrocyanic acid.

With a view to avoiding any risk of decomposition of the glucoside, we endeavoured at first to import fresh cassava roots from the West Indies, but it was found to be impossible to do this successfully, since the roots decomposed to a considerable extent in transit.

Recourse was then had to "bitter" cassava root, sliced in a fresh state and dried in the sun, and it was found that by this means material fairly rich in a cyanogenetic substance could be obtained. By preliminary experiments with this material it was found that the rind of the bitter root was much richer than the interior portion, and recently we have worked only with the rind prepared by stripping it from the fresh root of bitter cassava and drying it in the sun.

The whole of the material used in the present investigation has been obtained from the West Indies, and we are indebted to Sir D. Morris, Imperial Commissioner for Agriculture in the West Indies, who enabled us to obtain supplies in the first instance, and to Mr. Bovell, Superintendent of the Botanic Station, Barbados, who kindly undertook the preparation of the various consignments of dried bitter cassava root and rind we have received.

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* 'Annales du Jardin Botanique de Buitenzorg,' 1899, ii, vol. 16, p. 15.

Isolation of the Glucoside.

Estimations of the amounts of hydrocyanic acid obtainable from the dried sliced root and from the dried rind of bitter cassava were made by the method we have generally used for this purpose, viz., the complete extraction of the ground material with 90 per cent. alcohol and the hydrolysis of the glucoside contained in the residue left after distilling off the alcohol from this extract. This hydrolysis was accomplished by dissolving the residue in water and distilling the liquid almost to dryness after the addition of a few cubic centimetres of hydrochloric acid, the hydrocyanic acid in the distillate being titrated with silver nitrate solution by Liebig's method.* In this way it was ascertained that the dried sliced root yielded about 0·009 per cent. and the dried rind of the bitter cassava 0·035 per cent. of acid. These results agree fairly well with the quantities of the acid found by Francis, but the last is somewhat higher than those recorded by Carmody,† which ranged from 0·0113 to 0·0238 per cent. Owing to the impossibility of importing fresh roots, we have not been able to make any useful determination of the amount of acid obtainable from fresh roots of "sweet" and "bitter" cassava or from different parts of such roots, but we understand that investigations of this kind are now being carried out in India by Dr. J. W. Leather, Government Agricultural Chemist.‡

For the isolation of the glucoside the finely-ground cassava rind was completely extracted by percolation with 90 per cent. of alcohol. The solvent was distilled from the extract, the syrupy residue slightly diluted with water, filtered from the precipitated resinous and oily matters, and the filtrate decolorised by adding lead acetate, filtering out the precipitated lead compound of the colouring matter, and removing the excess of lead from the filtrate by treatment with sulphuretted hydrogen. This purified extract was then evaporated almost to dryness under reduced pressure at the ordinary temperature. The light brown syrup so obtained showed no tendency to crystallise even after long standing. It was therefore dissolved in alcohol and the solution poured into excess of ether. The matter precipitated by the ether consisted principally of dextrose. The decanted liquid was again evaporated nearly to dryness, the residue dissolved in alcohol, and the precipitation with excess of ether repeated. After each operation the solution was evaporated to dryness and the residue set aside so that crystallisation could occur, but it was only after this tedious process of dissolution in alcohol, precipitation by excess of ether, and evaporation to a syrup had been repeated five times that a crystallisable residue was obtained.

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* Compare 'Phil. Trans.,' 1901, B, vol. 194, p. 515; 1902, A, vol. 199, p. 399; 'Roy. Soc. Proc.,' 1904, vol. 72, p. 285.

† *Loc. cit.*

‡ Annual Report of the Imperial Department of Agriculture, 1905.

The difficulty of separating the glucoside from these residues appears to be due to the saccharine and extractive matters present, and it is only by the practically complete removal of these impurities by precipitation from alcoholic solution with excess of ether that the glucoside can be induced to crystallise. This process is both tedious and wasteful, the greater part of the glucoside being included in the several uncrystallisable fractions obtained when the solutions in alcohol are poured into excess of ether.

The crystalline residue eventually obtained was recrystallised from alcohol until colourless and of constant melting point. It crystallised in the spreading rosettes of colourless needles which are characteristic of phaseolunatin, had the same cool, bitter taste, and, like it, was readily soluble in water, less so in alcohol, and almost insoluble in dry ether. It melted at 138° (corr.). A mixture of phaseolunatin, prepared from the seeds of *Phaseolus lunatus*, and the cassava glucoside also melts at this temperature. There can be no doubt, therefore, that the glucoside of cassava is identical with phaseolunatin, which we have shown to be a dextrose ether of acetone cyanhydrin.

Hydrolytic Products of the Cassava Glucoside.

A portion of the purified extract, prepared as already described, was dissolved in water, a few cubic centimetres of 10-per-cent. hydrochloric acid added, and the mixture distilled almost to dryness. The distillate gave the Prussian blue reaction readily. To the remainder of the distillate, freshly-prepared lead hydroxide was added, and the mixture allowed to stand for some time, so that the hydrocyanic acid might be removed as lead cyanide. The filtrate from this was redistilled, and the first few cubic centimetres collected. To this were added a few drops of benzaldehyde, a similar small quantity of potassium hydroxide solution, and enough alcohol to dissolve the benzaldehyde added. On standing, the liquid deposited the characteristic crystals of dibenzylideneacetone (melting point 112°). The volatile hydrolytic products of the glucoside of cassava are, therefore, acetone and hydrocyanic acid, identical with those of phaseolunatin, affording further proof of the identity of the cassava glucoside with phaseolunatin.

The Enzyme of Cassava Root.

The enzyme was prepared in the usual way by extracting the ground dried rind of bitter cassava root with water previously saturated with chloroform, This liquid, when poured into an excess of alcohol, yielded a white precipitate of proteid matter, which, when dried by exposure to air on glass plates, formed slightly brown, granular masses. This preparation readily decomposed aqueous solutions of amygdalin and salicin, and also of phaseolunatin prepared from the seeds of *Phaseolus lunatus.*

The enzyme contained in the roots of the bitter cassava evidently closely resembles, and is probably identical with, the emulsin-like ferment obtained by us from the seeds of *Phaseolus lunatus,** and also from young flax plants (see this series, Part IV).

Cassava, therefore, like the other plants producing prussic acid which we have examined in the course of this investigation, contains a cyanogenetic glucoside, together with an enzyme capable of decomposing it. It is remarkable that the same glucoside, phaseolunatin, should occur in such different plants as *Phaseolus lunatus, Linum usitatissimum,* and *Manihot* species.

Although, for the reasons stated, we have found it convenient to employ the root of the bitter cassava for the isolation of the glucoside and its identification with phaseolunatin, there can be little doubt that this same glucoside occurs in sweet cassava, and that it is responsible for the production of prussic acid in that plant.

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* 'Roy. Soc. Proc,' 1904, vol. 72, p. 285.

The Action of Anæsthetics on Living Tissues. Part II.—*The Frog's Skin.*

By N. H. Alcock, M.D.

(Communicated by A. D. Waller, M.D., F.R.S. Received April 9,—Read May 10, 1906.)

(From the Physiological Laboratories of the University of London and St. Mary's Hospital Medical School.)

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I. Introduction.
II. Experiments—Series I.—Anæsthetics to inner and outer surfaces.
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V. Deductions. Relations to Part I. Alternative theories.

Introduction.

The currents given by the frog's skin and their variations after excitation both directly and through the nerves have already been studied by many authors,* but, as far as I can determine, no one has up to the present time observed the action of anæsthetics on the resting current, and as these pheuomena form a fitting sequel to those occurring in nerve (Part I of this series),† they form the subject of this paper. It is to be observed that from the nature of the object one of the possible alternative hypotheses in the case of nerve is here excluded, and it is therefore now legitimate to take a further step in explanation of the action of chloroform on the tissues generally.

Experiments.

Series I.

A chamber was constructed of vulcanite, through the bottom of which three non-polarisable electrodes projected. A movable partition of modelling wax, luted with moist china-clay, divided one electrode, A, from the other two, C and B (fig. 1). The skin was placed with the outer surface resting on A and C, the movable upper portion of the partition adjusted, and contact effected with the electrode B through a bundle of linen threads soaked in $m/10$ NaCl solution. In this series the electrode C served merely as a support, and the potential differences between A and B were alone considered.

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* Most of the references to the numerous papers on the frog's skin are to be found in the article by Waymouth Reid in Schäfer's 'Text-book of Physiology,' vol. 1, pp. 669—691. A criticism of the various theories is in the paper by Bayliss and Bradford, 'Journ. Physiol.,' 1886, vol. 7, p. 223. See also Waller, 'Signs of Life,' 1905.

† 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 267.

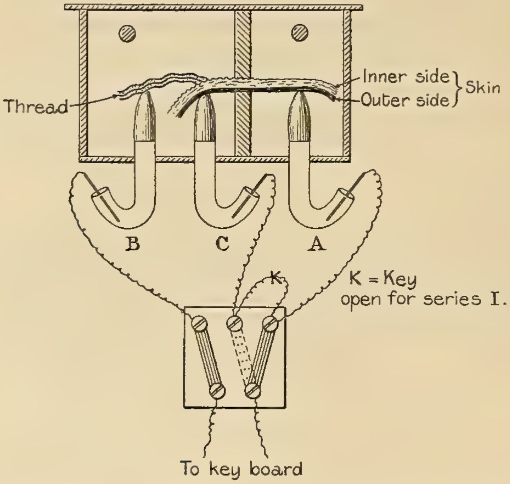

Fig. 1.—Chamber and Electrodes for Series I and II.

To make a preparation the frog is first killed by pithing, and the skin of the back cut into two longitudinal strips 3 to 5 mm. wide, one on either side of the middle line. These strips are very gently lifted up and the connections below severed, special care being taken to avoid stretching or other injury. A preparation of this kind gives a "resting" potential difference of from 0·0200 to 0·1100 volt,* sometimes exceeding this latter figure, the value varying according to the avoidance of injury and the condition of the frog, freshly caught frogs giving much higher values than those kept in captivity for some time. Any injury diminishes the potential indefinitely, and it is desirable to test the preparations from time to time by examining the skin *in situ*. If one electrode be placed very gently on the external surface of the skin and another on any portion of the subcutaneous tissue, one obtains the "resting" potential with the minimum of manipulation, and the value from the excised strip should not be much below this.†

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* Du Bois-Reymond ('Untersuchungen,' vol. 2, Abth. 2, pp. 9—20) gives a maximum value of 0·0561 volt and a minimum of 0·0044; Bayliss and Bradford (*loc. cit.*), 0·25 and 0·03 volt.

† There are many points still remaining to be determined with regard to the resting current in relation to variations in the conditions of observation: some of these are under consideration.

In the Experiments 502 to 506 inclusive, moist air containing about 16 per cent. of $CHCl_3$ vapour delivered from the apparatus previously described (Part I)* was applied *first* to the part of the chamber containing electrode B, which touched the inner surface, and *secondly* to electrode A, which touched the outer surface, the electrode not receiving $CHCl_3$ being ventilated with moist air. The figures plotted out in fig. 2 are the successive readings of the potentiometer balancing the E.M.F. from the skin, as in the previous experiments on nerve.†

It will be seen that $CHCl_3$ to B produces a minimal effect,‡ while $CHCl_3$ to A causes an immediate and very rapid fall to the zero line. In Experiments 500 and 501 (fig. 3) the order was reversed, $CHCl_3$ to A produced a fall of potential exactly as in fig. 2, $CHCl_3$ to B having again but a very slight action. The conclusion is therefore—

(i) $CHCl_3$ to the outer surface of the skin abolishes the current.

(ii) $CHCl_3$ to the inner surface of the skin has no action (neglecting the minimal effect noted above). From these conclusions follows another—

(iii) The apparatus giving the current is placed at or very near the outer surface.

Further inferences can be drawn; they are considered later.

Series II.

In Experiments 507 and 508 advantage was taken of the third electrode C which touched the outer surface opposite the electrode B, and measurements were taken by means of the ABC key of the potential between A and B, B and C, and A + C (in parallel) and B. The intervals between successive observations of the same pair of electrodes were about one minute, and the readings were in the order given, so that the readings for BC and (A + C) B are in each case a little later than those of AB. In both experiments all three readings showed the gradually lessening increase characteristic of a normal preparation under the conditions of experiment. $CHCl_3$ was then applied to the chamber containing electrodes BC.

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* *Loc. cit.*, p. 271.

† The figures have been corrected for electrode current; the error of reading is about ± 0·0001 volt. The potentiometer error is rather more, but as this is constant for all the experiments, no correction has been applied.

‡ This is probably due in part to a slight escape of $CHCl_3$ vapour to A, in part also because, although the outer surface has a high resistance (*vide infra*), this is not infinite.

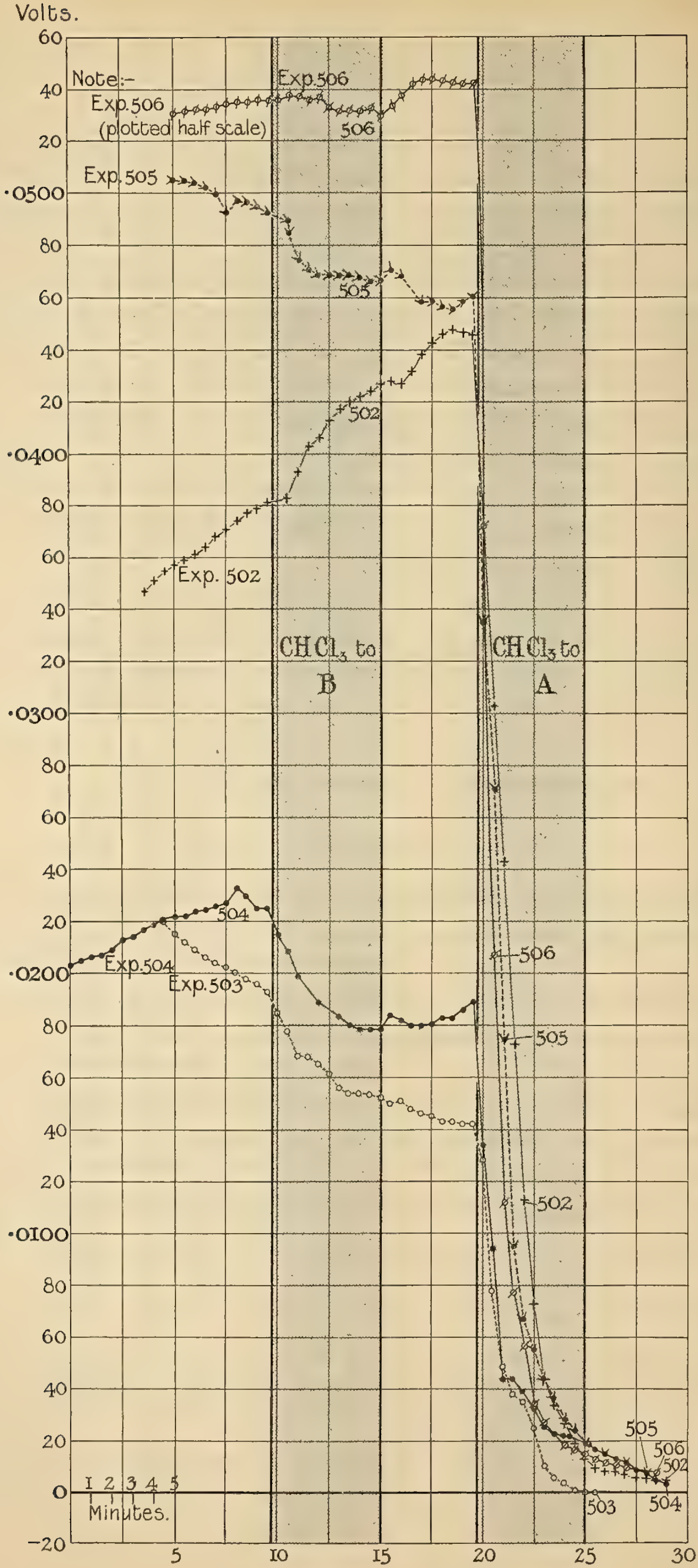

Fig. 2.—Series I. CHCl$_3$ (at approximately 16 per cent.) applied at the shaded parts. A = Outer Surface of Skin to Electrode; B = Inner Surface to Electrode.

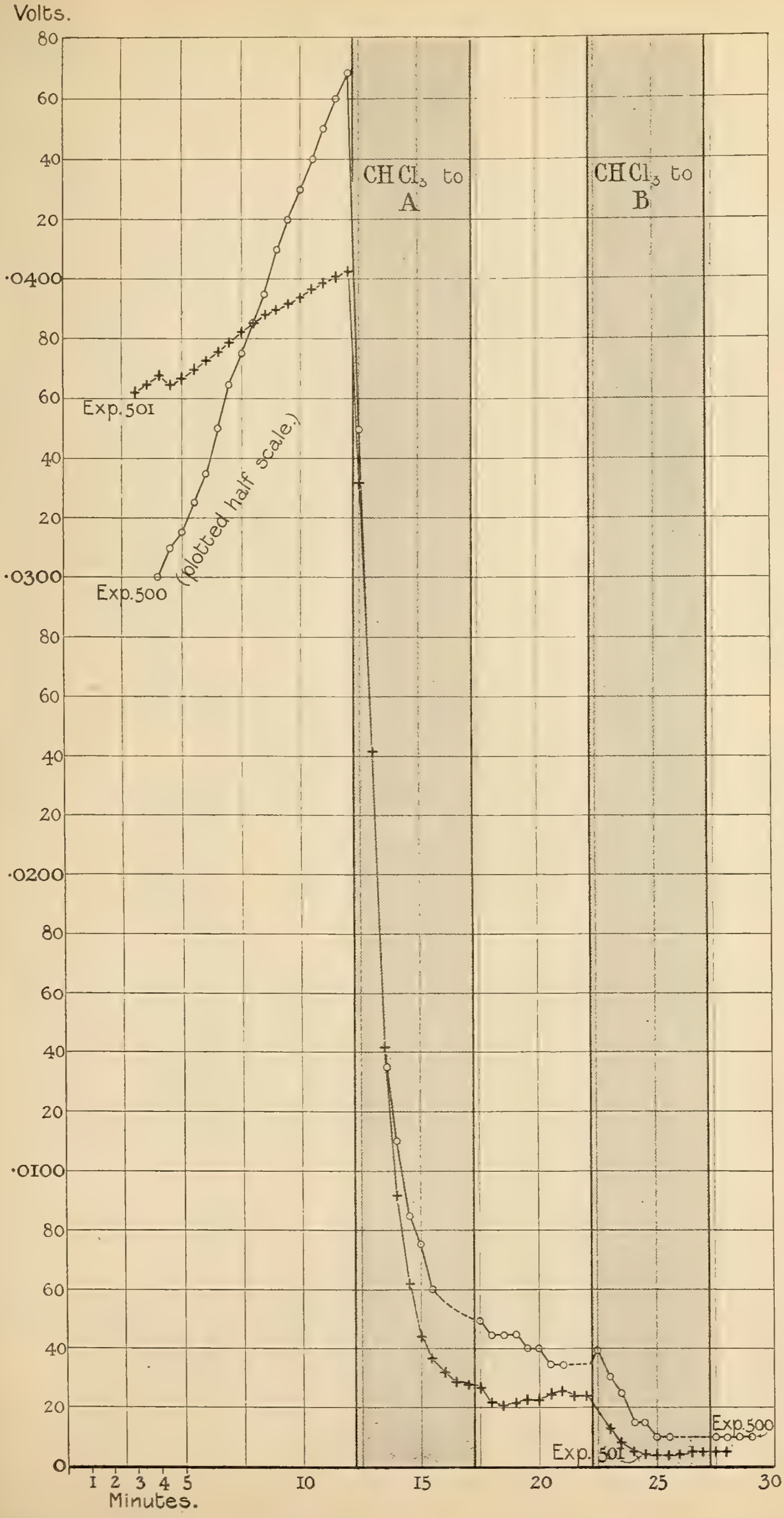

FIG. 3.—Series I. CHCl$_3$ (16 per cent.) to Parts of Skin. A = Outer Surface to Electrodes; B = Inner Surface. Experiment 501, CHCl$_3$ for 5-minute intervals; Experiment 500 for 3·5-minute intervals.

Experiment 507.

AB.	BC.	(A + C) B.
volt.	volt.	volt.
0·0299	0·0349	0·0338
355	350	354
370	361	369
382	354	366
390	357	369
396	358	372
	CHCl$_3$ to BC.	
410	+ 282	312
410	200	230
415	120	190
420	60	150
420	30	130
423	15	115
424	+ 5	110
426	− 2	102
	CHCl$_3$ off.	
434	− 8	95
437	− 10	93
437	− 11	91

Experiment 508.

AB.	BC.	(A + C) B.
volt.	volt.	volt.
0·0450	+ 0·0157	+ 0·0248
497	173	261
512	180	270
525	189	280
535	197	288
550	210	300
560	218	308
	CHCl$_3$ to BC.	
645	120	230
648	60	100 ?
670	30	180
683	13	167
687	+ 3	159
780	− 3	154
	CHCl$_3$ off.	
720	− 8	153

The sign + indicates that the current was directed from outer to inner in the skin.

Conclusions.—Chloroform applied to electrode BC—

(i) Causes no change in the potential between A B (as in Series I).

(ii) Abolishes the p.d. between B C.

(iii) Diminishes the p.d. between A + C and B.

For further observations see below.

Series III.

Determinations were made of the electrical resistance of skin before and after chloroformisation, in the same manner as the parallel series in nerve, the skin resting by its external on two non-polarisable electrodes in a chamber to which moist CHCl$_3$ vapour of about 16 per cent. or moist air could be passed at will. The probable error of a single observation does not exceed 2 per cent.

Conclusions.—Chloroform causes—

(i) A diminution of real resistance of about 24 per cent.

(ii) A diminution of polarisation of about 10 per cent.

The first of these conclusions contrasts markedly with the results of the experiments on nerve, where there was no alteration of the real resistance (as opposed to polarisation) within the limits of error of the method.

Skin, $CHCl_3$.

Experiment.	Resistance before $CHCl_3$.	T°.	Resistance during $CHCl_3$.	T°.	Resistance after $CHCl_3$.	T°.	R_3/R_1.	$CHCl_3$ per cent.
WHEATSTONE.								
	ohms.		ohms.		ohms.			
145	39,300	16·6	31,400	...	27,500	...	0·700	15
146	28,000	16·9	19,300	...	16,700	...	0·596	15
147	20,340	17·3	13,400	...	14,000	...	0·700	16·5
Mean	29,213	...	21,366	...	19,400	...	0·664	
KOHLRAUSCH.								
410 A	21,000	18·7	21,000	19·2	20,800	19·0	0·991	Control thread, M/10 NaCl.
B	18,500		15,100		13,500		0·730	Skin
411 A	19,300	19·0	19,000	19·4	19,000	19·1	0·985	Thread
B	18,700		16,700		14,700		0·768	Skin
412 A	18,300	18·9	18,300	19·5	18,000	19·2	0·984	Thread
B	12,701		11,400		10,200		0·803	Skin
413 A	18,300	19·3	18,300	19·7	18,300	19·3	1·000	Thread (same as 412 A).
B	17,400		13,700		13,400		0·770	Skin
Mean thread	19,230	18·98	19,150	19·45	19,030	19·15	0·990	Mean thread
Mean skin	16,830		14,230		12,950		0·765	Mean skin

Observations and Deductions.

It is to be observed that in these experiments there is no ambiguity (such as is possible in the case of nerve) as to the action of chloroform. Neither an increase or decrease of potential occurs (neglecting for the moment the very small residual effect noted above) when the drug is applied to the electrode B, in contact with the inner surface of the skin; this spot is therefore acting merely as a moist conductor. When the $CHCl_3$ is applied to the electrode A, which touches the external surface, the current is abolished. It is a necessary consequence that the apparatus giving the current is located at this spot.

This conclusion is in accord with all the classical observations on the resting current (see the authors quoted above); the only other observation that it is necessary to mention here is that of Waller* on the "blaze" current. Using the A B C method, he found that the seat of the electromotive effect was accurately localised at the spot on the external surface touched by the electrode, and that the opposite spot on the internal surface was quite inert. Although one cannot at the present time define exactly the relations existing between these "blaze" currents and the "resting" currents, there is no doubt that there is a close connection between them.

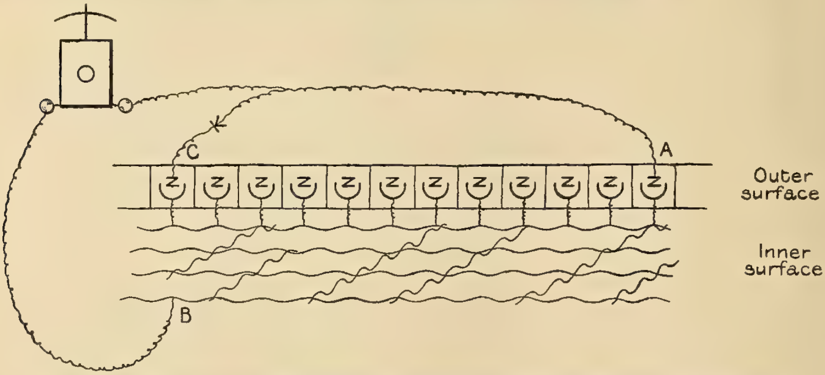

Fig. 4.—Diagram of Electrical Mechanism in the Skin of the Frog.

It is possible to make further deductions from the present experiments. If a diagram (fig. 4) be constructed to reproduce the observed phenomena, it must present the following features:—

(i) A series of separate cells placed side by side.

(ii) The positive elements (*e.g.*, zincs) lie towards the outer surface, the negative (*e.g.*, carbons) lie towards the inner.

(iii) The negative elements are connected with each other.

(iv) The positive elements are insulated.

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* Waller, *loc. cit.*

From the experiments in Series I, the postulate (ii) follows immediately; (i) and (iv) are necessary in this way. $CHCl_3$ abolishes the potential difference between the outer and inner surfaces, and renders that portion of the tissue isoelectric. If the whole outer surface was in electrical connection, the rendering isoelectric of any one part would cause a short-circuit there, and so diminish the current between any other point A on the outer surface and any point B on the inner surface—the experiments in Series I show that this is not the case. Series II confirms this conclusion. $CHCl_3$ to BC abolishes the potential difference there; it leaves unaffected AB, and the potential between A—C (in parallel) and B is diminished by an amount dependent on the relative resistance between A, B, and C. For a little distance inwards, therefore, the outer surface of the skin has a very high longitudinal resistance.

The postulate (iii) follows in a similar manner. Further, as the skin as a whole is a good conductor, and as the outer surface is a bad conductor, the inner surface must be a good conductor. As the negative elements are in connection with this tract, they are in connection with each other.

It is not yet possible to say what elements in the skin act in the manner of the (ZnC) cells in the diagram (fig. 4), still less the exact nature of the action. Certain parts of the frog's skin give an alkaline reaction, others an acid, and the relations between the secretion and the resting current (still more the variations on excitation) are yet to be determined.* But if one regards (for the sake of simplicity) only the glands, one can make a tentative picture of the process (fig. 5). The whole gland will resemble one cell in the diagram. As the carbons in fig. 5 are connected together and with the inner surface, so the lymph must bathe the inner surface of the gland and freely enter the cells of the gland. Now if the outer part of these cells—possibly

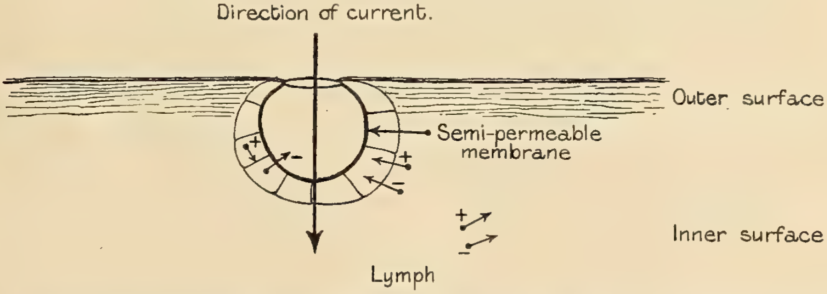

Fig. 5.—Tentative Diagram of Frog's Skin. For simplicity, the membrane (or part of the cell) has been drawn as permeable to – ions. In reality the conditions are more complex.

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* See Bayliss and Bradford, *loc. cit.*; also Hermann, 'Pflüger's Archiv,' 1878, vol. 17, p. 291.

the limiting membrane—has a directive or semi-permeable action, letting through some ions more freely than others, the general behaviour of the apparatus would approximate to the scheme. If, then, chloroform destroys the semi-permeable mechanism (perhaps in the manner suggested by Meyer[*] and Overton[†]) every sort of ion could wander freely in any direction, and so the whole tissue would become isoelectric. Further, this destruction would be equivalent to a diminution of viscosity, and so would account for the great increase in conductivity observed in Series III.

This must be regarded merely as a sketch of what is possible rather than a finished hypothesis; still this view presents certain problems in a manner that will admit of a solution which will either prove or disprove the suggestions here advanced.

At any rate, the experiments show:—

(1) That there are certain structures acting as semi-permeable membranes.

(2) That chloroform inhibits or destroys their functions.

Relations with Part I and Alternative Theories.

It will be remembered that in the consideration of the action of chloroform (and anæsthetics generally) on isolated nerve some phenomena were found which bear a relation to those now under consideration. Summarised they are as follows:—

(1) $CHCl_3$ acting on a nerve produces the same electrical effect as an injury.

(2) $CHCl_3$ produces less than 2 per cent. alteration in resistance.

If the hypothesis be adopted that there are in the axis cylinder of a nerve certain ions already present, it is plain that the action of the anæsthetic is the same on nerve as on skin, namely, that a semi-permeable apparatus is rendered completely permeable. It is not perhaps clear from the experiments on nerve alone whether this hypothesis is to be adopted or the alternative, that fresh ions are split off from the proteid by the action of the anæsthetic, but as it has been shown above that in the case of skin it is necessary to suppose the existence of a semi-permeable membrane, there is reason to suppose that this is the case in nerve also, and in fact it is not possible to create such a potential difference as is found in the case of nerve without some such arrangement, whether fresh ions are formed or no.[‡]

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[*] Meyer, 'Schmiedeberg's Archiv,' 1899 and 1901.

[†] Overton, 'Studien über die Narkose,' Jena, 1901, etc.

[‡] On the conditions under which ions can exist in the axis-cylinders of nerve-fibres, see Macdonald, 'Roy. Soc. Proc.,' vol. 76, B, p. 322, and Macallum and Menten, *loc. cit.*, vol. 77, B, p. 165.

It would appear therefore that this action of chloroform, namely, the breaking down of a semi-permeable apparatus, is the characteristic action of an anæsthetic on living tissue.

The additional possibility just mentioned remains to be considered. The view of Moore and Roaf * is that $CHCl_3$ combines with proteid, forming "unstable compounds or aggregates," and in doing so splits off inorganic ions, further, that "anæsthesia is due to a paralysis of the chemical activities of the protoplasm as a result of the formation of such aggregates." The present experiments (Parts I and II) give no direct information on these subjects, all that can be deduced with certainty is the conclusion given above. Moore and Roaf's theory may be adopted as an addition to this, or it may not. Whether or no this addition is justified by the experimental evidence adduced by these authors is a debatable point.

It is to be remarked that the observations (Part I, Series V and VI, Part II, Series III) on the electrical resistance of the tissues cannot be taken as proving or disproving an ion formation—they show that what is equivalent to a diminution of viscosity takes place (much in skin, little in nerve) and that in the latter case this diminution causes less than 2 per cent. alteration in conductivity, obviously a small production of ions would be masked by these other changes.† As the resistance and freezing-point determinations of Moore and Roaf were made with solutions, there were no membranes to cause this ambiguity, but from inspection of their figures it would appear that the mean differences, which they use in support of their argument, are less than the error of their experiments; it is only a matter of accident what values are obtained in such a case. The question is one that merits further study before a definite conclusion is arrived at.

I have again much pleasure in acknowledging the assistance of Dr. Waller, and I also wish to thank Professor Ayrton for his kindness in examining the electrical data and conclusions. Dr. Collingwood and Mr. Shapiro have again given me much help.

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* Moore and Roaf, 'Roy. Soc. Proc.,' vol. 73, p. 382, and vol. 77, B, p. 86.

† Using the apparatus mentioned in the text, it was found that any alteration in the resistance of *serum* was not detectable after $CHCl_3$, but that there was a diminution of approximately 15 per cent. in the case of blood (laked or simply defibrinated); it would seem that the effect of $CHCl_3$ on hæmoglobin would be worth investigating.

It would appear therefore that this action of chloroform, namely, the breaking down of a semi-permeable apparatus, is the characteristic action of an anæsthetic on living tissue.

The additional possibility just mentioned remains to be considered. The view of Moore and Roaf[*] is that $CHCl_3$ combines with proteid, forming "unstable compounds or aggregates," and in doing so splits off inorganic ions, further, that "anæsthesia is due to a paralysis of the chemical activities of the protoplasm as a result of the formation of such aggregates." The present experiments (Parts I and II) give no direct information on these subjects, all that can be deduced with certainty is the conclusion given above. Moore and Roaf's theory may be adopted as an addition to this, or it may not. Whether or no this addition is justified by the experimental evidence adduced by these authors is a debatable point.

It is to be remarked that the observations (Part I, Series V and VI, Part II, Series III) on the electrical resistance of the tissues cannot be taken as proving or disproving an ion formation—they show that what is equivalent to a diminution of viscosity takes place (much in skin, little in nerve) and that in the latter case this diminution causes less than 2 per cent. alteration in conductivity, obviously a small production of ions would be masked by these other changes.[†] As the resistance and freezing-point determinations of Moore and Roaf were made with solutions, there were no membranes to cause this ambiguity, but from inspection of their figures it would appear that the mean differences, which they use in support of their argument, are less than the error of their experiments; it is only a matter of accident what values are obtained in such a case. The question is one that merits further study before a definite conclusion is arrived at.

I have again much pleasure in acknowledging the assistance of Dr. Waller, and I also wish to thank Professor Ayrton for his kindness in examining the electrical data and conclusions. Dr. Collingwood and Mr. Shapiro have again given me much help.

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[*] Moore and Roaf, 'Roy. Soc. Proc.,' vol. 73, p. 382, and vol. 77, B, p. 86.

[†] Using the apparatus mentioned in the text, it was found that any alteration in the resistance of *serum* was not detectable after $CHCl_3$, but that there was a diminution of approximately 15 per cent. in the case of blood (laked or simply defibrinated); it would seem that the effect of $CHCl_3$ on hæmoglobin would be worth investigating.

Croonian Lecture, 1906.—*On Nerve Endings and on Special Excitable Substances in Cells.*

By J. N. Langley, F.R.S., Professor of Physiology in the University of Cambridge.

(Received and Read May 24, 1906.)

Amongst the fundamental problems of physiology is the determination of the actual seat of the changes which accompany or are the cause of physiological activity. The earliest step was the attribution of the more obvious functions of the body to the several organs which compose it. This has been followed by more or less successful attempts to ascertain the separate function of each tissue of an organ, and of each constituent of a tissue. And in recent years investigation has been largely concerned with the endeavour to localise functions long known, and those which from time to time are discovered, in the several parts, and in the several chemical constitutents of the ultimate unit, the cell.

I propose to-day to consider what is the actual seat of certain physiological activities in cells, and chiefly in those of muscular and nervous tissues. About 50 years ago Claude Bernard investigated the action of various poisons on the properties of the nervous and muscular systems. The main conclusions which he drew are still almost universally accepted. The most important observations were those on the action of curari. It had long been known that a muscle contracted when it was stimulated, but it was a question of interminable argument whether it responded to the stimulus in virtue of its own intrinsic properties, or in consequence of the presence of nerve-fibres in its substance.

Bernard discovered that after a small amount of curari had been administered to a frog, the motor nerves of the muscles of the trunk and limbs were no longer capable of causing contraction, but that the muscles contracted in the usual manner when stimulated directly. Assuming that the nerve-fibres in the muscles were as incapable of causing contraction as those outside them, it followed that the muscles are excitable in themselves, apart from any nervous impulses, and thus the first piece of fairly satisfactory evidence was obtained that muscle is not only contractile but irritable by other agencies than its nerves. Bernard* also showed that curari in the amount required to paralyse the motor nerve* did not paralyse either the sensory nerves or the central nervous system, or the trunks of the nerves near the central nervous system. He concluded that the primary poisoning effect of curari was due to a local action on that part of the nerve which lies in the muscle.

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* Pelouze and Bernard ('Comptes Rendus Acad. d. Sci.,' 1850, p. 533) were the first to point out that stimulation of the nerves had no effect in a frog to which curari had been administered. Bernard continued the investigation of the action of curari, and gave the

He pointed out also a similar local action on other tissues in the case of other poisons. Thus strychnine caused convulsions by an action on the central nervous system, but did not appreciably affect the properties of nerve-fibres or of muscles. And sulpho-cyanide of potassium destroyed the irritability of muscles—so that on stimulating them electrically, or pinching them or cutting them, no contraction could be obtained—but it had no marked action on either the central nervous system or the nerve-fibres.

Bernard's experiments thus distinguished a difference of behaviour in the nerve trunks, in the branches of the nerves in the muscle, and in the muscle itself, as well as (presumably) in the nerve cells of the central nervous system.

On the discovery of the nerve endings in vertebrate muscle, with their characteristic appearance in each class of animals, the paralysing action of curari on the peripheral branches of the motor nerves was naturally referred to the endings, and thus the nerve endings were taken to be different from the fibres from which they arose by division, and curari was said to paralyse nerve endings. This view was strengthened by the prevailing opinion that curari after prolonged action or in sufficiently large doses affected the nerve trunks also, for if the nerve endings were specially differentiated parts of the nerve-fibres it would be more reasonable to suppose that they would be specially affected by curari if the nerve-fibres were affected to some extent, however small, than if they were completely unaffected.

According to Bernard, curari acted first on the "terminations" of the motor nerves, and then, if in sufficient amount, gradually robbed the rest of the nerve of its irritability, beginning at the periphery and spreading towards the centre. A paralysing action on the nerve trunk was also found by Kölliker. Kühne† observed that in frogs recovering from slight curari paralysis the nerve first became effective near its end, and only later near its origin from the spinal cord. Later,‡ however, in experiments on the nerves close

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results in his lectures. Whilst these lectures were in the press ('Leçons sur les Effets des Substances Toxiques et Médicamenteuses,' Paris, 1857), and before Bernard's results were generally known out of France, Kölliker published the observations he had made on the action of curari (*cf.* 'Virchow's Archiv,' vol. 10, p. 3, 1856). His results agreed in general with those of Bernard.

* The phrase "paralysis of the nerve" is used in its customary sense to indicate that on stimulation it does not have its usual effect.

† Kühne, 'Müller's Archiv,' 1860, p. 477.

‡ Kühne, 'Ueber d. Wirkung des Pfeilgiftes auf die Nervenstämme,' Heidelberg, 1880.

to their endings in the muscles, he did not find that curari in large amount had any effect on them, and he was unable to reconcile this with his previous results. In fact, the question of the action of curari on nerves before they enter muscle is still one for discussion.

The view of the special character of the nerve endings has been further strengthened by histological observations on the changes occurring in the nerve endings under the influence of curari. Kühne described the living nerve endings in the muscle of lizards as having more distinct outlines after deep curari poisoning, and still more after slight curari poisoning and prolonged electrical stimulation of the nerves.* Miura† stated that prolonged (18 days) curari poisoning in the frog caused a dwindling in the size of hypolemmal fibres. Herzen and Odier‡ find that curari causes the hypolemmal fibres of the frog to become varicose, and that the axons of the nerve outside the muscle became covered with fine granules, the change decreasing towards the centre.

A variant of the general view was early put forward by Kühne. Kühne§ noticed that the irritability of the parallel-fibred sartorius muscle of the frog was not the same throughout. It was least at the ends, where, as he also found, no nerves or nerve endings were present. It was greatest near the point of entrance of the nerve. This, of course, might only mean that the nerve was more irritable than the muscle. But he found that curari, given in amount sufficient to paralyse the motor nerve to the muscle, did not abolish the difference in irritability between the middle and the ends of the muscle. This he considered could not be due to a difference in the muscle itself, since if he passed a constant current through the nerve supplying the muscle, the positive pole being near the muscle, so that the end of the nerve was thrown into an electrotonus, the irritability of the muscle became the same throughout. Consequently, he concluded that the terminal portion of the nerve, and possibly the whole nerve ending, was not paralysed by an amount of curari considerably greater than that required to block the passage of nervous impulses.

After a very large dose of curari, Kühne considered that the whole nerve ending was paralysed,|| although he obtained inconstant

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* Kühne, 'Verh. d. Naturhist.-Med. Vereins zu Heidelberg,' N.F., III, 1882.

† Miura, 'Virchow's Archiv,' vol. 105, p. 129, 1886.

‡ Herzen and Odier, 'Arch. Intern. d. Physiol.,' vol. 1, p. 364, 1904.

§ Kühne, 'Müller's Archiv,' 1860, p. 477.

|| According to Kühne, a saturated solution of sugar stimulates nerve but not muscle. The solution caused contraction only when applied to those parts of the sartorius muscle of the frog which contain nerve-fibres. This action was abolished by sufficiently deep poisoning with curari.

results as regards the equal irritability of the different regions of the muscle.*

On Kühne's view, then, curari readily paralysed a part of the nerve in the muscle a short distance before its ending; with an increasing dose of curari, the paralysis spread both down and up the nerve-fibres till finally the whole was paralysed; the proper irritability and contractility of the muscle remaining the same.

Some years later, Pollitzer,† working in Kühne's laboratory, confirmed the unequal irritability of different parts of the sartorius muscle, and found that the irritability corresponded with the number of nerve endings described by Mays in the different regions. He supported Kühne's view that the primary action of curari was not on the actual terminations of the nerves, and suggested that it might be on the cement substance of the last node, and not directly on either nerve ending or axis cylinder.

The difficulties in understanding the action of curari brought out by the work of Kühne and Pollitzer have had but little influence either on opinion or on the course of investigation, and I am uncertain whether Kühne himself adhered to his original view. Almost universally the simple and more intelligible theory has been taken that the axis cylinder of a nerve-fibre, in branching to make the nerve ending, alters its properties and is more susceptible to the action of various poisons.

A recognition of a difference in the properties of nerve endings and nerve-fibres has coloured the interpretation of physiological facts more and more as further facts have become known. Thus when the phenomena of fatigue were investigated, the nerve endings came to be considered as more liable to fatigue than the muscle, and as being different from nerve-fibres in which fatigue was produced with difficulty or not at all. The theory has also been applied to the connection of nerve-fibres with unstriated muscle, with gland cells, and more recently to the connection of nerve cells with one another. In all these cases, though to a varying degree, the nerve endings are commonly held to be especially affected by numerous poisons and as especially liable to fatigue. Lastly, in some cases the phenomena observed during the degeneration of a nerve have been attributed to the vitality of the nerve ending being greater than that of the axis cylinder of the fibre in the nerve trunk.

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* Sachs ('Reichert and Du Bois Reymond's Archiv,' 1874, p. 51) stated that, after curari in sufficiently large amount, the irritability of all parts of the muscle became the same. Pollitzer (*op. cit. infra*) found that maximal doses of curari lowered the irritability of all parts of the muscle, but that the curve of irritability of the several parts was like that of the normal muscle.

† Pollitzer, 'Journ. of Physiol.,' vol. 7, p. 274, 1886.

Now it cannot be denied that the great majority of the known facts receive a satisfactory explanation on this theory. Moreover, certain facts—those relating to the action of poisons on particular systems of nerves—are not easily explained on any other. Nevertheless, some recent observations go far to show that the theory is untenable. I will consider first the observations on motor nerve endings in striated muscle, since they are at present the most complete, and then briefly review those which concern other nerve endings.

Nerve Endings in Striated Muscle.

Nicotine is one of the many poisons which, in the usual acceptation of the phrase, paralyses motor nerve endings in vertebrates.

In some birds it has an additional action,* not so far observed in mammals. It causes certain muscles to pass into a state of tonic rigidity, and to remain in this state for many minutes. The contraction is not due to stimuli sent out by the central nervous system, for it occurs when all the nerves supplying the muscles are severed. If about 1 milligramme of nicotine, or any larger quantity, is injected into a vein of an anæsthetised fowl, the hind limbs, previously lax, gradually become stiff and extended (*cf.* figs. 1, 2, and 3), and they may remain so for a quarter to half an hour, or even longer.

When a graphic record of the contraction of a single muscle, such as the gastrocnemius, is made, the shortening of the muscle is seen to take place slowly and steadily to a maximum, which is approximately maintained for several minutes; this is followed by a gradual and much slower relaxation.

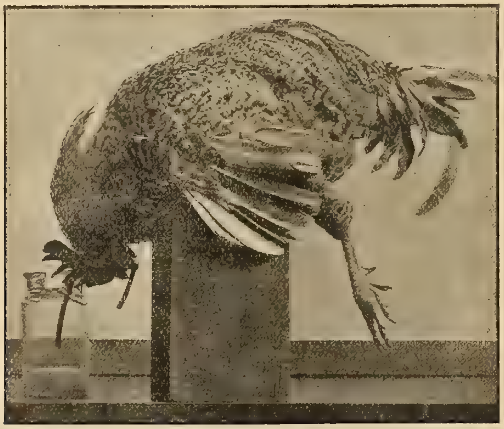

Fig. 1.—Fowl, anæsthetised with morphia and A.C.E. mixture, balanced on its thorax in a V-shaped piece of wood. The neck and legs hang down and are flaccid, the eyes are shut.

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* Langley, 'Journ. of Physiol.,' vol. 33, p. 380, 1905.

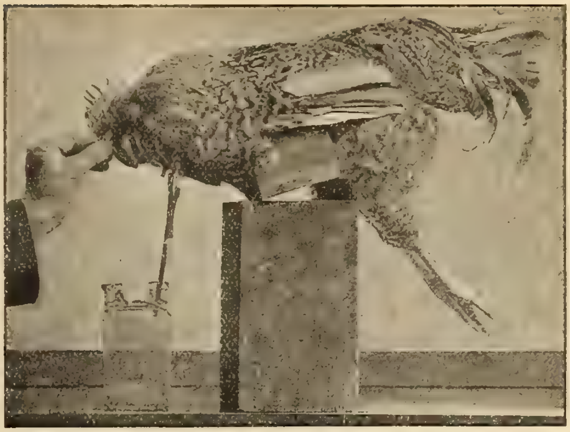

Fig. 2.—The same fowl as in fig. 1. Two minutes after injection of 5 milligrammes of nicotine into the jugular vein. The injection caused a gradual and fairly quick extension of the legs, retraction and twisting of the neck, and opening of the eyes. In order to show the eyes, the beak was held when taking the photograph. The fowl was unfastened throughout, and the injection caused no general movement nor any decrease of the anæsthesia.

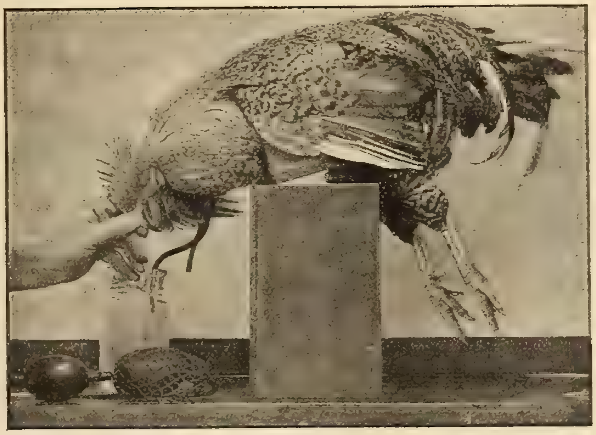

Fig. 3.—The same fowl half an hour after the injection of 5 milligrammes of nicotine. In the interval the fowl had been lifted up to observe the degree of contraction of the muscles, so that the position is probably not exactly the same as in the previous photograph. Most of the muscles still have some tone, more than they have in anæsthesia without nicotine ; but the eye muscles are apparently in the normal anæsthetic state, for the eye is closed. The eye began to close in about a quarter of an hour.

The rate of shortening varies with the amount of nicotine. After intravenous injection of 1 to 5 milligrammes the approximate maximum is not attained for 20 to 30 seconds; and even with very large amounts the rate is never comparable with that of the rapid twitch which is obtained by stimulating the nerve with the make or break of a galvanic current, or with a single induction shock. The contraction, in fact, is of a peculiar kind, not reproducible apparently by electrical stimulation of the nerve.

As I have already said, it is well known that nicotine has a similar effect to curari in so far as it prevents stimulation of a motor nerve from having any effect upon the muscle. In the fowl the paralysis is produced by 10 to 15 milligrammes of nicotine. The stimulating effect of nicotine occurs whether the amount given is sufficient to paralyse the motor nerves or no; amounts from about 0·5 milligramme upwards all cause contraction. Moreover, after the nervous effect has been abolished by 10 to 15 milligrammes, a subsequent dose will still cause muscular contraction, and by increasing the amount of successive doses contraction can be obtained a considerable number of times in succession, though the effect diminishes and at length ceases. This in itself is a curious fact,* for though it is extremely common for a drug to stimulate first and then paralyse, the recognised action of such drugs is to cease to stimulate after the paralysis has been produced.

The hypothesis which first suggests itself to account for this fact is that nicotine acts in different ways upon two different parts of the neuromuscular mechanism, paralysing the nerve endings on the one hand, and stimulating the muscle on the other.

It is clear that this view would be confirmed if curari had no effect upon the contraction caused by nicotine, for, as we have seen, curari is held to have an action on nerve endings but not upon muscle. But experiment shows that curari has a marked antagonising action on the contraction produced by nicotine; a sufficient dose of curari annuls the contraction produced by a small amount of nicotine and diminishes that caused by a large amount. The two poisons as regards muscular contraction are mutually antagonistic, though nicotine is the more powerful. This point is of fundamental importance, and I give the graphic records taken from one experiment in illustration of it.

Fig. 4 shows the contraction caused in a gastrocnemius muscle of the fowl† by 4 milligrammes of nicotine. Whilst the contraction was at its height, 50 milligrammes of curari were given. This it will be seen abolished or nearly abolished the contraction.

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* Some other alkaloids have a similar action; thus pilocarpin causes secretion, and slows the heart after it has paralysed the post-ganglionic nerves.

† The fowl was anæsthetised by morphia and A.C.E. mixture. The sciatic nerve was cut. The lever attached to the gastrocnemius tendon was weighted with 40 grammes. For other details of method, *cf.* 'Journ. of Physiol.,' vol. 33, pp. 381, 382, 1905.

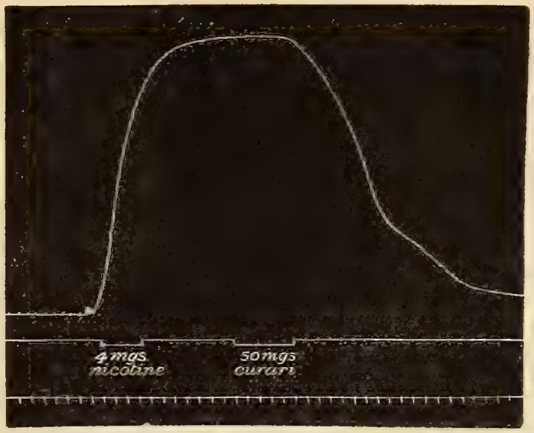

Fig. 4.—Abolition by curari of the contraction in the gastrocnemius muscle of the fow caused by nicotine. The lowest line marks intervals of 10 seconds.

The amount of curari given is two to three times the amount required to prevent the sciatic causing contraction of the muscle, nevertheless, the injection of 50 milligrammes of nicotine about three and a half minutes later caused the strong contraction shown in fig. 5, and this contraction in turn was reduced nearly to zero by a further injection of 50 milligrammes of curari* (fig. 5). A second injection of 50 milligrammes of nicotine 10 minutes later was almost without effect, the substance stimulated by nicotine being temporarily paralysed, but the muscle readily contracted on direct electrical stimulation.

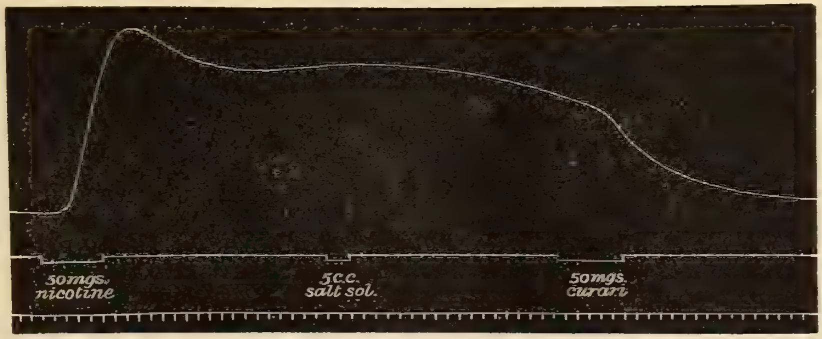

Fig. 5.—Continuation of tracing given in fig. 4. Mutual antagonism of nicotine and curari. The 5 c.c. of salt solution was injected to show that the injection itself did not cause relaxation. Time marked in 10 second intervals.

Experiment, then, with curari does not confirm the obvious view as to the mode of action of nicotine given above. It shows that if nicotine has an action on muscle, so also has curari. But some other form of experiment is required to decide whether nicotine does, in fact, act upon the muscle direct.

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* Large amounts of curari are apt to weaken or stop the heart, but control experiments show that the nicotine contraction continues unaltered for several minutes after complete cessation of the heart beat, and that the curari relaxation occurs when the blood pressure is unaffected by the injection.

It is clear that what is required is to examine the behaviour of the muscle when no nerve endings are present. A means to this end is to cut the nerve and allow it time to degenerate. It is known that after section, nerve-fibres degenerate up to their point of entry into the muscle fibres and it has been almost universally assumed that the nerve endings, which are but the terminal branches of the nerve, degenerate also. It is, however, as I have already said, not uncommonly believed that the nerve endings degenerate more slowly than the nerve-fibres, and some doubt has been expressed as to whether they degenerate at all.

This question must then first be examined. The degeneration of the nerve endings after section of the nerve-fibres has been taken so much as a matter of course, that there are, so far as I know, two papers only published on the subject.

The earlier investigations were made by Sokolow,* on the gastrocnemius muscle of the frog. He examined the muscle for nerve endings by the gold chloride method, 14, 28, 32 and 44 days after section of the sciatic nerve.

Fourteen days after section of the sciatic nerve, no change was observable in the nerve endings. In the other three cases, good staining of the muscle was apparently only obtained in that taken 32 days after section of the nerve, but it may perhaps be assumed that the changes described were observed, though less satisfactorily, in the other two. According to Sokolow, the different nerve endings undergo change at very different rates, so that in all cases some normal nerve endings remained. Those which were altered, but still visible, showed fine dark stained granules in a basis staining less than normal. The alteration was sometimes only at the ends of the hypolemmal fibres, and different hypolemmal fibres of the same ending showed different degrees of change.

In other cases, some or all of the hypolemmal fibres had completely disappeared.

In the latter type, the nerve-fibre with fragmented myelin was traced up to the muscle and Sokolow states that the absence of the nerve ending was not due to imperfect staining. In some instances he describes the spaces left by the nerve endings as being filled with a fine deposit of gold.

Although these observations leave something to be desired, they do, I think, show that the nerve ending undergoes first granular degeneration and then absorption. A point which raises some doubt in my mind is a brief statement by Sokolow that in all cases contraction was still obtained by stimulating the nerve. It seems unlikely that after nerve section some of the nerve-fibres and nerve endings should be normal, whilst others had so far degenerated that the nerve endings had completely disappeared, or that the conductivity of the nerve should last as long as 44 days.

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* Sokolow, 'Archives de Physiol.,' 1874, p. 300.

The second published work on this subject is by Huber.* His observations were made on the interosseus muscles of the rabbit, the posterior tibial nerves being crushed to interrupt continuity. The nerve endings were stained with methylene blue. On the second day after the nerve crushing, some of the hypolemmal fibres showed round or oval thickenings staining deeply with methylene blue, these rapidly increased in number, and when the great majority were in this state, stimulation of the nerve had no longer an effect. At a later stage, up to six days, no staining of the nerve endings was obtained, but at times there was a faint staining of the sole. Huber, however, lays no great stress on this result, since the staining in the normal condition was uncertain. At a still later stage (about 30 to 178 days), *i.e.*, when time was allowed for regeneration, nerve endings were again obtained.

The only gap in these observations is that the intermediate forms between the first stage of granular degeneration and absorption are not described. They show definitely that in mammals the nerve ending alters rapidly on nerve section, losing its normal staining power with methylene blue, and it may fairly be inferred that the nerve ending, like the axis cylinder, degenerates.

In order to form an independent opinion on the question, I have made some observations on the nerve endings of the frog by the methylene blue method. In the sartorius muscle of the frog a considerable number of nerve endings can with certainty be shown by methylene blue; I have not had one failure in more than a hundred trials. I have cut on one side, under anæsthetics, the branch of the sciatic (n. descendens communis) which supplies the sartorius muscle, and a variable time after the section, killed the frog, taken the sartorius muscle of the cut and uncut side and treated them in the same way in methylene blue. I find that about three weeks after section of the nerve, the nerve endings show deep stained small granules in a faintly stained basis, that later the granules became smaller and fewer, and the basis barely visible. About six weeks after section, no trace of nerve endings is to be seen. A semi-diagrammatic sketch of one of the granular nerve endings I give in fig. 6.

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* Huber, 'Amer. Journ. of Physiol.,' vol. 3, p. 339, 1900.

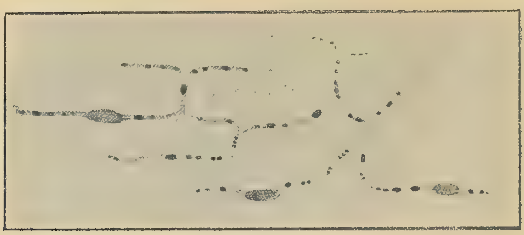

Fig. 6.—Nerve ending in sartorius muscle of frog three weeks after nerve section. Methylene blue stain. The tint of the several parts of the nerve ending in the figure represents roughly the degree of stain, but the tint of the hypolemmal fibres, and, to a less degree, that of the nuclei, is too deep.

Lastly, Tuckett has investigated the nerve endings in the flexor profundus muscle of the pigeon, by the methylene blue method. He finds that the hypolemmal fibres show granular degeneration in two days or less, and that after three days they disappear. The details of his work will be published shortly.

As the results of these various observations, we may then, I think, safely conclude that after section of a nerve, the nerve endings undergo first granular degeneration and then absorption.

And, if this be granted, it is clear that we can determine whether nicotine does or does not act on muscle by administering it a sufficient time after a motor nerve has been cut.

I have examined* the reaction of the gastrocnemius muscle of the fowl, 6, 8, 27, 38, and 40 days after section of the external peroneal nerve which innervates it. In all cases a contraction was obtained essentially similar to the normal nicotine contraction, the only difference apparently observed being an increased response to small doses of the poison, indicating an increased excitability after denervation, such as has been described in some cases of unstriated muscle after nerve section.

Since the contraction is certainly not decreased, and may be increased, by degeneration of the nerve endings, the nicotine contraction must be produced by an action on the muscle and not on the nerve endings.

Passing now to the effect of curari. On the fowl's gastrocnemius muscle, after degeneration of the nerves, curari is still capable of reducing the nicotine contraction. The only difference is that it is—so far as the experiments go—a less powerful antagonist; a fact probably related to the apparent increased excitability to nicotine already mentioned. Curari, then, must also have an action on the muscle substance.

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* *Op. cit. supra.*

The mutual antagonism of nicotine and curari on muscle* can only satisfactorily be explained by supposing that both combine with the same radicle of the muscle, so that nicotine-muscle compounds and curari-muscle compounds are formed. Which compound is formed depends upon the mass of each poison present and the relative chemical affinities for the muscle radicle.

Since the formation of the nicotine-compound causes contraction, and that of the curari-compound does not, it is obvious that the chemical re-arrangements set up in the muscle molecule by the combination of one of its radicles are different in the two cases. In fact, it seems probable that a special radicle is necessary for the combination with a number of chemical bodies, and that the compound formed leads to further change depending upon the nature of the compound.

Having then arrived at the conclusion that both nicotine and curari combine with some substance in the muscle, we have to consider whether there is any reason to suppose that they combine with some substance in the nerve ending also. The only action that can be attributed to them is the paralysing action.

There is, unfortunately, no direct and conclusive evidence either one way or the other. But as the ascertained action of the poisons on muscle is sufficient to explain their paralysing action, it is unnecessary to resort to the assumption of an additional effect on nerve endings. It is a common action of drugs first to stimulate and then to paralyse, so that there is plenty of analogy for the view that nicotine, after stimulating, paralyses the constituent of the muscle on which it acts.

Curari, as we have seen, decreases the irritability of the muscle to the nicotine stimulus, and it is reasonable to suppose that it decreases also the irritability of the muscle to stimuli arriving by the nerves. I conclude, then, in terms of the theory given above, that the compounds which the poisons form with the muscle are less irritable and conductive than the normal muscle substance.

The continuation of the stimulating effect of nicotine after nervous impulses is rendered ineffective, we may explain on the supposition that the nicotine muscle compound ceases to be irritable or conductive to nervous stimuli, before the maximal combination with nicotine has taken place. The degree of combination is essentially a question of the relative chemical affinities of the radicle of the muscle substance with other radicles and with nicotine.

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* Pal ('Centralb. f. Physiol.,' 1900, p. 255) found that curari nerve-paralysis could be more or less completely abolished by physostigmine. The matter was further worked out by Rothberger ('Arch. f. d. ges. Physiol.,' vol. 137, p. 117, 1901), who also showed (*ibid.*, vol. 92, p. 398, 1902) that several substances, and amongst them nicotine, can, in proper conditions, partly restore irritability to motor nerves paralysed by curari. In writing my earlier account (*op. cit. supra*) I was unaware of Rothberger's observations. The physiological antagonism in this case is very incomplete, a fact which is in harmony with the theory of antagonism of poisons I gave some years ago ('Journ. of Physiol.,' vol. 1, p. 367, 1878).

It is true that, as mentioned earlier (p. 172), microscopic changes have been described in nerve endings as the result of giving curari, but these do not necessarily show a special action on the nerve ending. In the instance described by Kühne, the increase in distinctness in the nerve endings of the lizard might equally well be due to an action on the "sole" or muscular protoplasmic mass below the nerve ending. And with regard to the observations of Herzen and Odier it is to be noted that the granular changes found in the nerve endings after curari were also found in the axis cylinders a variable distance up the trunk of the nerve, and it is certain that the abolition of nerve effect, produced by small and by moderate amounts of curari, is independent of any changes in the trunk of the nerve.

Further, there is no certain histological difference between the nerve-fibres before and after branching into nerve endings; although some differences have been described, the general evidence is that the nerve endings are simply branches of the axis.

The probability then, I take it, is that none of the phenomena of nerve and muscle stimulation are due to a chemical difference between the axis cylinder and the nerve endings, and in that case it follows not only that the poisoning phenomena of a large number of drugs are due to changes brought about directly in some constituent of the muscle, but also that the peripheral fatigue usually attributed to changes in the nerve endings is really due to fatigue of a special constituent of the muscle.

Since neither curari nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curari is not identical with the substance which contracts. It is convenient to have a term for the specially excitable constituent, and I have called it the receptive substance. It receives the stimulus and, by transmitting it, causes contraction.

Beyond this we cannot go at present with any certainty. I may indicate briefly one or two possibilities.

It is well known that Bottazzi* has given reasons for the theory that sarcoplasm is contractile, but that it contracts more slowly than the fibrillar material. We might then refer the nicotine contraction to the sarcoplasm, and the slowness of the contraction would imply that the fibrillæ are not stimulated at all by nicotine. Since the contraction caused by a single electrical stimulation of the nerve is a quick contraction, it would naturally follow that it is due solely to the fibrillæ. Thus one or other of the two contractile substances would come into action alone. But if the stimulating action of nicotine is solely in the sarcoplasm, we should naturally conclude, in accordance with the argument given above, that its paralysing action is also solely on the sarcoplasm. In this case we should have to conclude that the sarcoplasm normally transmits the stimulus caused by a single electrical stimulation of the nerve without itself contracting, but does not transmit the stimulus caused by nicotine though it does itself contract. This conclusion presents great though perhaps not insuperable difficulties.

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* Bottazzi, 'Journ. of Physiol.,' vol. 21, p. 1, 1897.

If, in consequence of these difficulties, we take the view that the nicotine causes contraction of the fibrillæ and not of the sarcoplasm, there are two possibilities: the receptive substance may be part of the sarcoplasm, or it may be a radicle of the contractile molecule.

It might be urged in favour of the former view that according to Kühne the nerve ending is in some cases completely separated from the fibrillæ by sarcoplasm. On the other hand, even if a thin layer of sarcoplasm does intervene between nerve and muscle, it does not necessarily follow that it takes any essential part in the passage of the stimulus.

On the latter view it is clear that the contractile molecule must either have at least one receptive radicle in addition to that affected by nicotine and curari, or it must be capable of direct stimulation. The hypothesis, however, demands that the stimuli passing by the nerve cannot affect the contractile molecule except by the radicle which combines with nicotine and curari. And this seems in its turn to require that the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by du Bois Reymond.

Lastly it is to be noted that whether the receptive substance is part of the sarcoplasm or part of the fibrillæ, it remains to determine whether the receptive substance is localised in the immediate neighbourhood of the nerve ending or exists throughout the length of the muscle fibre. The observations of Kühne and Pollitzer (quoted above) on the different irritability of the nerve-containing and nerve-free parts of the muscle afford some evidence for, at any rate, unequal distribution, but the main question can, I think, only be settled by experiments on the effect of local application of nicotine such as I have to mention presently.

In the preceding statement, the view that curari and a number of other poisons act on muscle has been placed in sharp antagonism with the view that they act on nerve endings. But the possibility of an intermediate view must be mentioned. There are some observers who consider that nerve and muscle are continuous, and it is conceivable that a certain region of the junction should belong to both nerve and muscle in the sense that either could keep it alive. The special properties attributed to the nerve ending might then be attributed to the junctional region, half muscle and half nerve. With regard to this, it will be noted that the junctional region, if it exists, must be in the muscle, since we have good reason to believe that all the visible nerve ending degenerates on section of the nerve.

In the simplest case, that of the nerve endings of amphibia, the substance which has been described as continuous with the endings is clearly the sarcoplasm of the muscle, which stretches throughout the muscle fibre, and no substance of distinct histological characters which could be regarded as a junctional substance is shown by the microscope.

In the end plate form of nerve ending there is it is true the specially modified sarcoplasm which forms the "sole." But, as was shown by Kühne, some of the hypolemmal fibres commonly run past the limits of the sole.

But the theory of continuity seems to me to have the balance of evidence decidedly against it. As Kühne* pointed out in a previous Croonian Lecture, the properly stained hypolemmal fibres terminate usually in rounded ends sharply marked off from the muscle substance, and every method which brings out the nerve endings clearly, brings out this characteristic. The outline of a hypolemmal fibre is as distinct at its end as it is in its pre-terminal course. In the case of typical nerve endings, it is only when they are viewed in all the indistinctness of the fresh specimen, or are made granular by treatment, that any appearance of continuity is suggested.

Kühne has also pointed out that excitation of the muscle substance does not cause a stimulus to pass back through the nerve ending, and this might reasonably be expected to occur if there were continuity.

The chief observations in favour of continuity are those on the development of the nerves in the embryo.† As I have said elsewhere, if it were shown that continuity exists in the early embryonic state, it would not follow that it continues to exist in later stages. With differentiation of function, histological separation might well take place.‡

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* Kühne, 'Roy. Soc. Proc.,' vol. 44, p. 427, 1888.

† For a critical account of these observations, *cf.* Kölliker, 'Zeitschr. f. wissensch. Zool.,' vol. 82, 1905.

‡ Since the above was written, a paper has been published by Pflüger ('Arch. f. d. ges. Physiol.,' vol. 112, p. 1, 1906), in which he upholds the theory of continuity. It is a measure of the need for further investigation that the well-known observations on the

The preceding argument has been based chiefly on the fact that nicotine excites certain muscles in the fowl, as well as abolishes, when given in sufficient amount, the effect of the nerves upon them.

Now, as mentioned already, nicotine abolishes motor nerve action in all vertebrates investigated. It would be very remarkable if the stimulating action occurred only in birds. I have not made any systematic investigation of the question, but, in thinking about it, I remembered that v. Anrep* had described catalepsy of the fore limbs of the frog as being caused by nicotine after destruction of the spinal cord and that the phenomenon had been confirmed by Dickinson and myself† and additional details given.

The tonic contraction produced in the muscles of the fore limb of the frog‡ is clearly similar to, though not identical with, that produced in the gastrocnemius and other muscles of the fowl. Its duration, however, may be much greater. The effect is to cause the fore legs to be drawn over the sternum in the manner described by early observers, who, however, did not definitely attribute it to peripheral stimulation.

An illustration of the effect of nicotine on the fore limbs is given in fig. 7. The brain and spinal cord of a frog were destroyed; in this state the muscles are, of course, flaccid, and the limbs if raised and let go, at once fall. The frog was placed on its back with the fore limbs by the side of the body. Then a little 1 per cent. nicotine was injected into the abdominal cavity; this caused the assumption of the position shown in fig. 7.

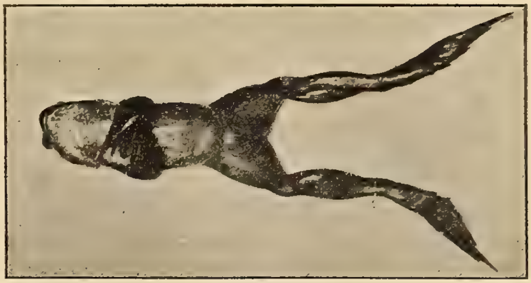

Fig. 7.—Frog. Killed by destroying the whole of the central nervous system. Contraction of the muscles of the fore limbs caused by nicotine.

If a graphic record of the movement of the limb is taken, it is seen that the

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tissues of vertebrates which he quotes seem to me, as to many others, without exception inconclusive. Of the tissues of invertebrates I have but slight experience.

* v. Anrep, 'Arch. f. (Anat. u.) Physiol.,' 1879, Supp., pp. 167, 209.

† Langley and Dickinson, 'Journ. of Physiol.,' vol. 11, p. 265, 1890.

‡ Most of my experiments were made during the breeding season, when it is possible that the tendency to tonic contraction is greater.

maximum flexion may not be attained for several minutes. Such a record is given in fig. 8.

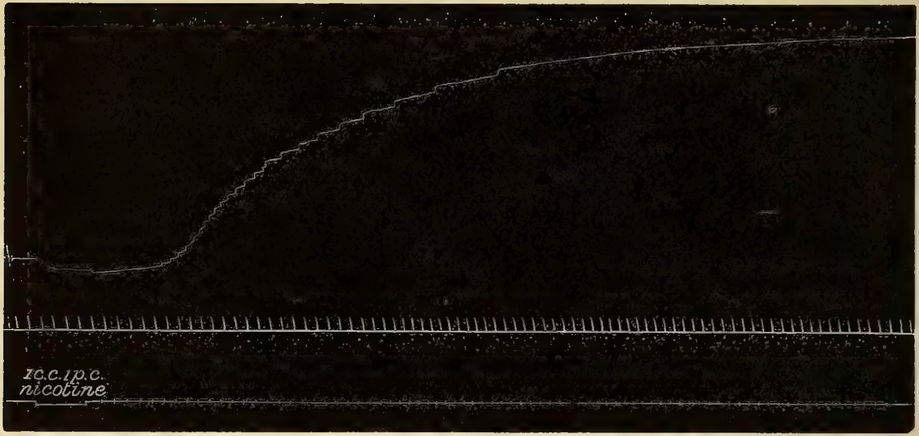

FIG. 8.—Frog. Brain and spinal cord destroyed. A thread was tied to the manus and connected with an unweighted lever, so that flexion of the arm caused a rise of the lever,* 1 c.c. 1 per cent. nicotine injected into the abdominal cavity at the time shown by the signal. Time marked in 10 seconds.

The tonic contraction is not confined to the muscles of the fore limb. If dilute nicotine (0·05 per cent.) is injected into the bulbus arteriosus, it is seen that all the muscles of the body, except those of the thigh and lower leg, become tonically contracted. The duration, strength, and other features of the contraction vary in different muscles, and in some instances it is accompanied by fibrillar twitching. In the thigh and lower leg muscles there is, so far as can be seen with the eye, twitching only, if the nicotine is sufficiently dilute. Moreover, if instead of injecting nicotine it is applied locally to the muscles (0·05 per cent. solution and upwards) local contraction is produced; thus the numerous extensors and flexors of the toes of the fore and hind limb can be made to contract separately. It may be noted, too, that the rate of contraction of the abdominal muscles is considerably quicker than that of the flexors of the arm. Different muscles, then, show nearly all the possible variations.

An interesting point is that on applying nicotine to a portion of a muscle the tonic contraction occurs in this portion only and does not spread to the rest of the muscle. When stronger solutions of nicotine (0·5 to 1 per cent.) are used, tonic contraction also takes place in the thigh muscles. On application to a portion of a muscle, a local wheal, like the ideo-muscular contraction, is formed at the place of application. If the sartorius muscle is cut out and excess of fluid removed, local contraction at the ends of the muscle where no nerves are present is obtained by local application of 1 per cent. nicotine, the contraction, however, is less strong than that produced by 1 per cent. nicotine to the part of the muscle which does contain nerves. If this contraction is a genuine nicotine contraction like that produced in other muscles by injection into the circulation of small amounts of nicotine, it shows that the nicotine-receptive substance is not confined to the nerve endings. But further experiments are required before it can be said that these similar contractions are identical.*

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* It will be noticed that the flexion takes place in a series of small jerks; the cause of this I have not yet investigated.

I have not had time to make more than a few experiments on the effect of curari on the nicotine contraction. So far it results that nicotine will still cause contraction after the nerves have been paralysed by curari, but that a much larger amount of nicotine is required. Whilst there is a mutual antagonism between the two poisons, curari is a much more potent antagonist of nicotine than it is in the fowl.

Nicotine has a similar action on the muscles of the toad, and there is a similar mutual antagonism between the action of nicotine and curari on the muscles. In the toad the contraction of the flexors and extensors of the arm is equal or nearly equal, so that there is little or no movement.† But the cataleptic condition is usually much more marked than in the frog, and affects the leg as well as the arm, though to a less extent. The fore limbs can be moved about almost as if made of lead, and stay with but slight return movement in any position in which they are placed consistent with the arrangement of the joints and ligaments. In fig. 9 I give a photograph of a toad in which the brain and spinal cord had been destroyed, and 1 per cent. nicotine then injected into the abdominal cavity. The limbs soon became cataleptic, and the photograph is taken of one of the positions in

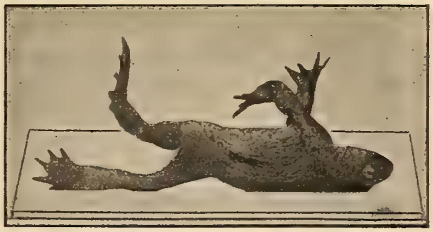

Fig. 9.

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* Nicotine is strongly alkaline, and I have not yet tried the effect of alkalies. Strong curarisation does not abolish the action of 1 per cent. nicotine; but this may be due to the nicotine being in sufficient amount to overcome a curari effect.

† I have not tried the effect of nicotine on the male toad during the breeding season.

which the limbs were placed. It is noteworthy that this cataleptic condition is abolished by a sufficient dose of curari.

Nerve Endings in Nerve Cells.

Evidence of a similar nature to that which I have given for the relation of motor nerves to striated muscle exists also for the relation of the nerve-fibres which run from the spinal cord (pre-ganglionic fibres) to end in connection with sympathetic nerve cells.

The action of nicotine on sympathetic nerve cells* is very similar to that which it has on the gastrocnemius muscle of the fowl. It stimulates nearly all the sympathetic nerve cells of the body, and in larger dose, varying in different cases, it abolishes the effect of stimulating the pre-ganglionic nerves, *i.e.*, it paralyses the nerve-fibres.

Curari in large amounts paralyses the pre-ganglionic fibres, the amount required varying with different classes of sympathetic fibres; when it does this it prevents a normally effective amount of nicotine from causing any stimulation.

Now nicotine has as strong a stimulating effect on the sympathetic ganglia after degeneration of the pre-ganglionic fibres as it has normally. There is no suggestion of the existence of any structure in connection with the nerve endings which could keep them intact after section of the nerves from which they arise, and I conclude that they degenerate after nerve section.

Ramón y Cajal, Dogiel, Huber, and others, who have used either Golgi's method or Ehrlich's methylene blue method for staining nerve endings, find that the nerve-fibres running to a sympathetic ganglion are not continuous with the nerve cells, but end free in close contact with them. I have obtained similar results by the methylene blue method. Anderson ('Journ. of Physiol.,' vol. 28, p. 499, 1902) gives one piece of experimental evidence for continuity. He finds that in very young animals delay of development of myelin in the cervical sympathetic nerve-fibres occurs not only on section of the nerve itself, but also on section of the post-ganglionic fibres of the superior cervical ganglion. The latter fact, he considers, shows continuity through the nerve cells of pre- and post-ganglionic fibres. It is certain, however, that developing nerve-fibres are much more influenced by injuries than the fully formed ones; and it is possible that the atrophy of the nerve cells caused by cutting the post-ganglionic fibres in very young animals injures the nerve endings in close contact with them, and in this case the section of the post-ganglionic fibres is practically equivalent to section of the pre-ganglionic fibres.

Some evidence against continuity is given by the fact that nicotine applied to a ganglion causes no axon reflex; *i.e.*, the stimulus set up in the nerve cells does not pass back to the nerve-fibres. How much weight is to be attached to this depends upon the nature of the nervous impulse about which at present there is doubt. But the fact is, I think, fairly conclusive against the continuity theory put forward by Bethe;[*] for if conducting neuro-fibrils traversed the nerve cell, stimulation of the neuro-fibrils in the cell either directly or indirectly must lead to nervous impulses being conducted in both directions. And that the pre-ganglionic fibres can conduct in both directions is shown by the axon reflex produced on electrical stimulation of the nerves.

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* *Cf.* Langley, 'Journ. of Physiol.,' vol. 27, p. 224, 1901.

Nicotine, then, stimulates the nerve cells. Since curari in sufficient amount prevents this action, curari also must act upon nerve cells, and we may infer, as in the case of striated muscle, that neither act upon the nerve endings.

It may be noted that there is histological confirmation of the direct action of nicotine on nerve cells, for, according to Cosmettatos[†] it produces chromatolysis of the cells of the superior cervical ganglia of the rabbit, a change which Eve[‡] showed is not produced by protracted stimulation of the cervical sympathetic.

One piece of evidence is lacking in the case of nerve cells, viz., that they retain their fundamental properties after nicotine or curari poisoning in the way striated muscle does. The nerve cells cannot be stimulated apart from the nerve-fibres these give off, so that it is only by analogy that I conclude that the substance affected by the poisons is a special receptive substance and not the fundamental substance of the cell.

The experiments on sympathetic ganglia give, I think, good ground for believing that in the central nervous system also, both the specific effect of nicotine, strychnine, and other poisons, and the phenomena of fatigue must be attributed to an action on nerve cells (either root cells or short fibred commissural cells) and not to an action on nerve endings. They support the view that the special functions of the central nervous system are carried on in vertebrates by the nerve cells (including their dendrons), and not by a neuro-fibrillar network outside the nerve cells.

Nerve Endings in Unstriated Muscle and Glands.

The nerves under this heading are post-ganglionic fibres arising from the sympathetic and allied ganglia.

The evidence centres round the action of adrenalin. Adrenalin was discovered comparatively recently,[§] and it does not stand on the same footing as curari and nicotine, for from the first it has been a matter of discussion whether it acts on nerve endings or on muscle. Since it has long been recognised that poisons act upon cells apart from nerve endings, the discussion on the action of adrenalin did not for a time raise the general questions we are considering here.

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[*] Bethe, 'Allg. Anat. u. Physiol. d. Nervensystems' (Leipzig), 1903.

[†] Cosmettatos, 'Archives d'Ophthalmologie,' 1904.

[‡] Eve, 'Journ. of Physiol.,' vol. 20, p. 334, 1896.

[§] Oliver and Schäfer, 'Journ. of Physiol.,' vol. 18, p. 230, 1895.

Taking the facts so far as they are now ascertained,* it may be regarded as certain that the numerous effects produced by adrenalin are no whit impaired, and probably, indeed, are increased by degeneration of the sympathetic nerves which supply the structures on which it acts. On the assumption that the degeneration of the nerve endings is accompanied by degeneration of the nerve-fibres, this is clear evidence that adrenalin stimulates by acting directly on unstriated muscle and gland cells.

The assumption is based on the fact that the nerve-fibres, on section, degenerate up to the peripheral plexus (or network). There is no obvious reason why the degeneration should not proceed throughout. Some cells, it is true, occur in close connection with the peripheral plexus, but it is practically certain that these are connective tissue cells. A few experiments have been made by Fletcher† on the nerve plexus in the retractor penis of the hedgehog. In these he found that the nerve plexus did not stain with methylene blue after degenerative section of the nerves supplying the muscle.

But there are difficulties in accepting the conclusion that adrenalin does not act on nerve endings which are not met with or are met with to a much less extent in dealing with the effect of nicotine and curari upon striated muscle and nerve cells. The difficulties depend upon the apparently specific relation of adrenalin to the sympathetic system. The broad facts with regard to this relation are as follows :—

Certain parts of the body are innervated by two systems of nerves, viz., by the sympathetic system and by either the cranial autonomic or the sacral autonomic system. It was pointed out by myself‡ that the stimulating effects produced by adrenalin are in all cases (with the doubtful exception of the pupil in the dog)§ like those produced by stimulating the sympathetic, and not like those produced by stimulating either of the other systems.

The sympathetic nerves cause contraction of nearly all the arteries of the body, but the contraction varies in ease of production and in intensity;

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* *Cf.* Lewandowsky, 'Arch. f. (Anat. u.) Physiol.,' 1899, p. 360; Langley, 'Journ. of Physiol.,' vol. 27, p. 237, 1901; Brodie and Dixon, *ibid.*, vol. 30, p. 500, 1904; Elliott, *ibid.*, vol. 33, p. 401, 1905; Langley, *ibid.*, vol. 33, p. 376, 1905.

† Fletcher ('Proc. Physiol. Soc.,' p. xxxvi, 1897), 'Journ. of Physiol.,' vol. 22.

‡ Langley, 'Journ. of Physiol.,' vol. 27, p. 237, 1901.

§ The contraction of the pupil caused by adrenalin has been shown by Elliott (*op. cit. supra*) to be due to central stimulation.

the effect of adrenalin is, in general, proportional to the effect of electrical excitation of the nerves. The arteries of the lungs, according to Brodie and Dixon,* and the coronary arteries, according to Schäfer,† are equally unaffected by electrical stimulation of the sympathetic nerves and by adrenalin; the like absence of effect is found with many of the veins of the body.

The sympathetic nerves supplying a given organ may produce different effects in different animals. In all these cases, so far as observed, the effect of adrenalin corresponds with the effect of the nerves. This has been shown in mammals chiefly by Elliott,‡ and in amphibia and fish by Bottazzi.§

The differences which are found between the effects of adrenalin and of electrical stimulation of sympathetic nerves are, first, that in a tissue which receives both motor and inhibitory nerve-fibres from the sympathetic, the balance of motor and inhibitory effects are not necessarily the same with the two stimuli; thus, in some cases adrenalin produces much greater and more lasting inhibition than can be caused by electrical nerve stimulation, and, secondly, that some tissues are readily affected by stimulation of the sympathetic nerves, and barely at all, or only in enormous doses, by adrenalin.

Furthermore, it has been shown by Dale|| and confirmatory evidence has been given by Elliott, that ergot abolishes all the constrictor effects of the sympathetic, without impairing any of the effects of the cranial or sacral autonomic nerves, or the inhibitory effects of the sympathetic. In this state adrenalin, according to Dale and to Elliott, causes inhibition in many of the regions innervated by the sympathetic, and has no constrictor effect whatever. Thus, for example, the bladder of the ferret normally contracts when either the sympathetic or the sacral nerves are stimulated, or when adrenalin is injected. After ergot the sacral nerves cause contraction as before, the sympathetic nerves and adrenalin cause inhibition.

The special relationship of adrenalin to the sympathetic system is, *primâ facie,* strongly supported by the close developmental connection of the suprarenal body with the sympathetic ganglia. This argument, however, loses some of its force in consequence of Dale's discovery that ergot, which is not formed at all in the body, has also a special action on the tissues innervated by the sympathetic system.

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* *Op. cit. supra.*

† Schäfer, 'Archives Intern. de Physiol.,' vol. 2, p. [141], 1904.

‡ Elliot, *op. cit. supra.*

§ *Cf.* Bottazzi and Costanzi, 'Nuove Ricerche sull' Azione dell' Adrenalina e d. Paraganglina' (Napoli), 1905.

|| Dale, 'Proc. Physiol. Soc.,' p. lviii ('Journ. of Physiol.,' vol. 32) and 'Journ. of Physiol.,' vol. 34, in the press.

The difficulty, then, which we have to face is that of reconciling the fact that adrenalin has a special relation to tissues innervated by sympathetic nerves, with the deduction from the degeneration experiments that adrenalin does not act on the nerves or their endings. Elliott, adopting the theory that nerve and unstriated muscle are continuous, considers that adrenalin acts on the junction of the two—the myo-neural junction—and does not act either on muscle or on nerve endings.

It is important to keep clearly in mind that the question whether adrenalin does or does not act on muscle or on nerve endings is, up to a certain point, a matter of nomenclature.

If the nerve, the myo-neural junction, and the muscle, are regarded as successive parts of a continuous mass, it may then logically be said, on this theory, that adrenalin acts on the myo-neural junction and does not act on muscle or on nerve. But the conclusion depends upon the definition of muscle and of nerve. The junction, since it is not regarded as a separate entity comparable to the muscle cell or the nerve cell, must consist either of muscle substance or of nerve substance, or of both. That is to say, adrenalin must act either on muscle substance or on nerve substance, or on both. And as muscle substance is a part of the muscle cell, and a nerve substance is a part of the nerve cell, it cannot, from this point of view, be properly asserted either that adrenalin does not act on muscle, or that it does not act on nerve. The legitimate statement from the premises is that it does not act on any muscle substance or on any nerve substance outside the limits of the myoneural junction.

Two arguments which have been used against the view that adrenalin acts on muscle should, I think, be definitely discarded. Dixon, Brodie and Dixon, and Elliott argue that since, after apocodeine, adrenalin does not stimulate certain unstriated muscle, and barium chloride does, adrenalin cannot act on muscle. This is the traditional argument used with regard to the effect of curari on nerve endings in striated muscle. It rests on the identification of the contractile molecule of muscle with the whole muscle substance. It is not only conceivable that the irritable substance should be part of the sarcoplasm, but also that it might be a radicle of the contractile molecule. And in either case the irritable substance might be put out of action without destroying the contractile power of the muscle.

Elliott argues also that it is unlikely that the blood vessels of the lungs or heart should differ in their intrinsic musculature from those of the intestine to such a biochemical degree that the one set should be slightly dilated by adrenalin and the other powerfully contracted. But, in fact, there is variation, and this can only be explained by a variation in nerves or muscles, and the variation might theoretically take place as well in the muscle as in the nerve.

As regards the localisation of the receptive substance, strong evidence that this occurs to a considerable extent is afforded by the action both of adrenalin and of chrysotoxin on tissues which have a double nerve supply, but the evidence cannot be regarded as conclusive.

In order to account for the special relation of adrenalin to the sympathetic system, Elliott supposes that the excitable substance of the myo-neural junction is formed in consequence of the union of the nerve with the muscle and not in virtue of any inherent property of the muscle. The central point of this theory is clearly applicable whether there is union or only contact of nerve and muscle, and there is much that is attractive in the view that the receptive substance owes its formation to the nerve, but it does not, I think, satisfactorily account for the different degree of action of adrenalin and chrysotoxin on the receptive substance in different cases,* nor for the persistence of the substance after nerve degeneration. If the receptive substance were formed in the muscle solely in consequence of its connection with a nerve, it seems most unlikely that the muscle would be able to take on the formation when the nerves have disappeared.

The view I take is that the relation of poisons in general to special systems of nerves depends upon the developmental history of the connection of the different systems with the particular tissues. It seems to me certain that the various cells of the body have a constant tendency to vary in chemical composition, and it is probable that these variations in any one tissue are approximately the same at the same time.† It is fairly certain that different systems of nerves establish nervous connections with the cells at different periods of phylogenetic development, and it is probable that when nervous connection is made it tends to make permanent certain of the chemical conditions existing at the time.

Thus the different systems of efferent nerves would chiefly, at any rate, owe their differences to the different characters of the "receptive" substances of the cells with which they have become connected.‡

General Conclusions.

In the foregoing account we have seen reason to believe that in each of the three great types of connection of the peripheral end of an efferent nerve with a cell it is some constituent of the cell substance which is stimulated or paralysed by poisons ordinarily taken as stimulating or paralysing nerve endings. Reasons, though less complete, have been given for supposing that these poisons have no special action on nerve endings, and that physiologically the nerve ending is not essentially different from the nerve-fibre. In that case not only the function of reacting to numerous chemical bodies, but probably also the special liability of both afferent and efferent nerves to fatigue must be transferred from the nerve endings to the same constituent of the cell.

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* *Cf.* Langley, 'Journ. of Physiol.,' vol. 33, p. 408, 1905. In this paper I have also considered the action of some other alkaloids which bear on the question, such as those of Anderson on the action of atropine, pilocarpine, and eserine on the denervated pupil.

† A difference in the time of development of the sympathetic nerves to the skin and of those to the viscera might account for the different degree of action of poisons on the tissues innervated by the two sets of nerves.

‡ Some inferences from this theory I have given in the paper quoted above.

This theory adds to the complexity of the cell. It necessitates the presence in it of one or more substances (receptive substances) which are capable of receiving and transmitting stimuli, and capable of isolated paralysis, and also of a substance or substances concerned with the main function of the cell (contraction or secretion, or, in the case of nerve cells, of discharging nerve impulses). So far as this is concerned, it does but accentuate a view which has often been put forward and which indeed in some form or other is inseparable from the idea of protoplasm.

I have spoken of different "substances" in the cell with the intent to use as vague a term as possible. The "substances," I take it, are radicles of the protoplasmic molecule. At present, however, I do not think it advisable to speculate further either on this question or on certain other questions raised by the conclusions arrived at in the paper. There are a number of obvious experiments still to be made, and these, it may be hoped, will settle some of the problems, the solution of which is now but guess work.

The Experimental Analysis of the Growth of Cancer.

By E. F. Bashford, M.D., J. A. Murray, M.B., B.Sc., and W. H. Bowen, M.S., F.R.C.S.

(Communicated by Professor J. Rose Bradford, F.R.S. Received May 30,—Read June 14, 1906.)

In the present paper we shall attempt to analyse the growth of cancer when propagated artificially in mice, mainly on the basis of 25,000 inoculations of Jensen's tumour performed in conjunction with Dr. W. Cramer on behalf of the Imperial Cancer Research Fund; but also with reference to inoculations made with 32 other mouse tumours during the past three years. Although the question of the continuous or interrupted nature of cancerous proliferation is of fundamental importance, both from the standpoint of the ultimate explanation of the nature of the disease, and from the standpoint of its treatment, such an analysis has never been attempted before. It has been assumed that the growth of cancer is vegetative, as inexplicable as any other form of growth, only to be partially understood by an elucidation of the processes by which normal tissues become cancerous. Artificial propagation enabled us to submit this assumption to the test of experiment, and imposed the necessity of determining by direct observation whether propagated cancer exhibited a mode of growth throwing light on the nature of the disease and the apparently continuous proliferation of sporadic tumours. While the experimental propagation of cancer may reveal new facts with a bearing on the nature of the disease it also affords opportunities for rational and empirical therapeutic experiments, and adequate opportunity for controlling the results. These two purposes have been constantly kept in view in our investigations.

When a number of animals are inoculated with a transplantable mouse tumour, all do not develop tumours and the tumours which do develop are not all of the same size after the same interval. In order that propagated cancer might be available for the second of these purposes it was necessary to find out what influence the variable conditions of experiment exerted on the proliferation of the cells. In the course of these preliminary studies facts bearing on the nature of cancer have also been ascertained.

Irregularities in the rate and amount of growth are introduced by (1) Transference from one race of mouse to another even when nearly allied: (2) Transference from young to old mice of the same race or *vice versâ:* (3) Variations in the site of implantation of the cancerous tissue: (4) Variations in the amount of the tissue implanted and in the manner of introducing it: (5) Variations in the character of the tumour cells themselves. Any one of these factors may cause a very great deviation from the rate and amount of growth observed previous to the subinoculations introducing it, and invalidate the results of experiments of which information as to possible modification of growth was the object. The variations depending on the first four factors mentioned must be eliminated before variations can be referred to the tumour cells themselves.

We have taken the following precautions in studying the fluctuations which we believe depend on differences in the tumour cells.

1. The same race of mice has been used throughout. We have observed differences in the suitability of animals of different colours even among the ordinary English tame mice; and we have avoided the use of those varieties prized by mouse-fanciers. The wild mouse probably offers more uniform conditions than the tame mouse, but a sufficient stock of uniform age is difficult to obtain, keep and supervise. Jensen's tumour rarely yields a number of successful subinoculations in wild mice equal to that obtained in a control batch of tame mice, and this result when once obtained has not been maintained, but is followed by an increasing difficulty of propagation. The experiments in wild mice may be looked on as control observations to those recorded in tame mice.

2. The tame mice used have been of uniform age, and from five to seven weeks old. We showed that young animals provide conditions more favourable for the artificial propagation of cancerous tissue than old animals. This conclusion has been amply confirmed by our later experience, and in one of its aspects also by the work of Ehrlich and Apolant,* who state that the age of the animals is without importance and, especially, that old females are not more suitable than young animals for the propagation of mammary tumours. We have found that the greater suitability of young animals is even more marked than we at first suspected. The inoculation of a tumour into young and old animals respectively may occasionally give similar results in the two cases, or even a less favourable result in young mice, still such results are exceptional in our experience. As a rule, a much higher percentage of tumours develops in young animals, and they attain large dimensions in a shorter time after inoculation. The tumours which have developed most rapidly, *e.g.*, attaining a weight of 1·05 grammes in a mouse of 9 grammes, within five days, and those ultimately attaining the largest dimensions as compared with the size of the host, have always occurred in young mice, although tumours of 7 or 8 grammes also develop rapidly in adult animals. Slow growing tumours, which remain of relatively small dimensions, occur both in

* 'Berl. Klin. Wochenschrift,' No. 28, July, 1905.

old and in young animals. The extent to which the youth of the animals usually favours the continuation of growth after transplantation may be illustrated by the results of 18 series of inoculations, in which portions of the same parent tumours were transplanted simultaneously into young and adult animals respectively; 214 implantations into adult animals three to six months old yielded 62 tumours, or 29 per cent. were successful; 363 implantations into young animals five to seven weeks old gave 172 tumours, or 47 per cent. were successful. This result is by no means an extreme case, either as regards proportion of successes or as regards difference in age of the inoculated animals.

3. When the precautions above indicated are observed, the individual variations in the general suitability of different mice of the same race and age are negligible if implantation be performed in the same site, provided sufficiently large numbers are used. We have preferred the subcutaneous tissue of the back. The attempt to perform collateral series of intra-peritoneal inoculations was abandoned, owing to the frequency with which growth within the peritoneum had occurred secondarily by extension from tissue implanted in the abdominal muscles.

4. We have endeavoured to transplant pieces of healthy-looking tissue of uniform size by means of hypodermic needles, and have obtained more satisfactory results by this method than by breaking tumours down into an emulsion and injecting larger quantities of tissue suspended in physiological salt solution. With certain reservations the rate of development and the size the daughter tumours will attain within 10 days is directly proportionate to the amount of healthy tumour tissue implanted; 0·02 to 0·03 gramme of tissue usually gives larger tumours within a given time interval than 0·005 to 0·01 gramme.

5. When the conditions referred to in the four preceding paragraphs are maintained uniform, fluctuations independent of them appear, and we shall endeavour to show that they are, in all probability, natural features of proliferation. The detailed study of these fluctuations has been undertaken with the tumour which has proved readily capable of transmission during the longest period yet attained, viz., that of Jensen. This tumour has now been propagated for four and a-half years, without permanent alteration in its histological characters or its behaviour. We have obtained success in from 5 to 90 per cent., occasionally even in 100 per cent., of the animals inoculated, the percentages being based on data obtained from those mice which were still alive* 10 days after the inoculations were made. The amount of tissue

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* It is our practice to kill from time to time a number of mice during the first 10 days after inoculation, in order to examine the site of implantation. Tumours of transplant-

transplanted in each animal varied between 0·01 and 0·02 gramme.* The pieces were selected from the whole tumour, and hence their behaviour furnishes an estimate of the proliferative energy of its component parts. The use of a restricted number of random fragments is rendered necessary, because it is impossible to transplant the whole of every tumour; the number of animals required of itself limits the investigation.

The method of experimental propagation by implanting minute cellular grafts leads to a progressive subdivision of the parenchyma, and to the distribution over a large number of animals of the descendants of cells previously associated together in one animal. The experimental tumours consist of a parenchyma arranged in alveoli. The study of the early stages after transplantation shows that, at first, single alveoli constitute separate centres of growth, and we may therefore term them the "parent alveoli" of the tumour. Since the cells of different alveoli do not intermingle, the progeny of the discrete growing centres in the transplanted tissue remain separate, and are further separated from one another as the parent alveoli increase in size and bud off daughter alveoli at the surface. When minute portions of such a tumour containing very numerous daughter alveoli are in turn transplanted it is very improbable that any one fragment will contain cells from each parent alveolus, *i.e.*, progeny of all the primary growing centres in the cellular graft which gave rise to the tumour. On the contrary, such a fragment is likely to contain cells closely related to one another, *i.e.*, from only one of the new growing centres of which the tumour is ultimately composed. Thus, in the course of repeated implantation, the tumours obtained come to represent less and less all the constituent cells of any entire tumour in the preceding transplantations made during the long-continued experimental propagation. In order that they should do so it would be necessary to mix homogeneously all the tumours obtained at each series of implantations. The purposes of our investigation were fulfilled by the method of repeated subdivision and isolation. By this method a repeated analysis of the power of growth of small groups of cells and their descendants can be obtained. The limited number of centres of growth represented in any single graft can

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able size, 0·75 to 1·5 grammes weight, are rare before 8 to 10 days. As the object of these experiments was to estimate the power of continued growth as distinct from mere transitory proliferation, some such time limit was necessary. Estimates of the percentage of success and of the frequency of the spontaneous cessation of growth in tumours which had established themselves must exclude transitory proliferation of the cells introduced and inflammatory swellings at the site of inoculation.

* The weight of the fragments inoculated into each animal has been arrived at by weighing the mass of tumour used for transplantation and dividing by the number of animals used, *e.g.*, 1 gramme of tumour transplanted into 100 mice gives 0·01 gramme per implantation.

be better appreciated by considering also that they have been obtained as the result of a triple process of selection; firstly, the rapidly growing tumours of a batch have been selected because of the greater powers of growth exhibited; secondly, only the healthy parts were used for transplantation; and thirdly, a further sifting has been effected by the elimination of those cells which degenerate after transplantation. Taken together with the simultaneous reductions of the number of cells continuing growth at each fresh implantation, the repeated implantation of minute cellular grafts renders it practically impossible that any one tumour at the present stage of propagation should still contain cells representing all the growing centres of a tumour even two or three transplantations antecedent to it.

The percentage of tumours developing after transplanting is, however, only one means of measuring the proliferative power of a tumour experimentally.* It is an arbitrary measure selected for its convenience of application. It merely records that, of a number of fragments taken from a tumour, a certain proportion grew and the remainder were absorbed after implantation in fresh animals. It necessarily neglects variations always obtaining in the weights of tumours in every batch. It is obvious that a sporadic tumour may be obtained, or a time may come in the course of the prolonged selection of tissue for implantation in the future continued propagation of Jensen's tumour when all transplantations will yield tumours. Should this ever be so, measuring the energy of growth by the percentage of tumours developing would fail to reveal any fluctuations. The fluctuations in percentage of success which had previously occurred would retain their importance, and a different method of measurement might still reveal fluctuations dependent on the same factors as great as those represented in these experiments by percentages of success varying between 5 and 100 per cent. of the animals used.

We have studied the growth of the tumour in parallel series of experiments at different times. In order to compare the results we have estimated the percentage of success attending the subinoculation of all tumours transplanted. The repeated subdivision of the transplanted tumours results in the separate propagation of many strains, which become increasingly numerous as time goes on, spreading out like the branches of a tree from any tumour selected as a starting point. On the basis of this relation a genealogical tree of all the transplanted tumours has been constructed in which the intervals of time between successive transplantations are also recorded. This result is achieved by measuring the number of days since propagation commenced and marking the respective dates of transplantation, so as to mark off abscissæ; the power of proliferation being measured by marking the percentage of successful implantations as ordinates. The point, determined by these two variables for every tumour transplanted, records its power of proliferation and the date of transplantation.

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* The weights which the tumours attain in equal times present great fluctuations as well. In series with a high percentage of success many tumours attain a weight of 1 gramme in the course of 10 days, while series with low percentage of success seldom show tumours of 0·5 gramme weight in the same time. This may be due to the greater number of cells continuing growth in each animal in series of high percentage, and therefore does not necessarily indicate a more rapid rate of proliferation of the individual cells. For this reason we have not been able to use the weight of tissue produced in a given time as a means of comparing proliferative power at different times.

When the point so obtained from any one tumour at the end of the series is connected by a line with the point similarly obtained for the tumour from which it was derived, and the connections followed backwards through the corresponding points of the preceding transplantations, the absolute duration of propagation and the steps in the lineage of the tumour at the end of the series can be seen at once. As the process of connecting up the points is continued backwards the lines from the points obtained for other strains converge and coalesce till all ultimately unite in the point obtained for the percentage of success attending the primary transplantation of the sporadic growth.

The graphic records accompanying this paper are small portions of a large chart recording in this way the results of all our experiments with Jensen's tumour extending over a period of two and a-half years.

For the purpose of recording the experiments each batch of implantations performed with one tumour is labelled with a number, stating the number of successive transplantations from the beginning of the series. For example, the parent tumour of a batch of implantations belonging to the 40th transplantation has been obtained after 39 successive transferences to fresh mice. To distinguish between several batches, the parent tumours of which have been obtained after the same number of transferences, a letter of the alphabet is added to the number of the transplantation. The genealogy of the various series of implantations is not indicated by this nomenclature, and for this purpose the graphical records, now to be described, have been devised.

As it is important that the exact meaning of this graphical record should be clearly understood, the method by which it is built up may be exemplified by a special case (fig. 1) forming part of another chart (fig. 2).

(1) On the 739th day of propagation, a tumour of Transplantation 45, Series C, was transplanted into 52 mice which were labelled 46 C; 32 mice died in the first 10 days after transplantation. In the remaining 20,

11 tumours developed; *i.e.,* 55 per cent. of the implantations were successful.

(2) On the 751st day of propagation, a second tumour of Transplantation 45, Series C, was transplanted into 30 mice, the experiment being labelled 46 F. No deaths occurred in the succeeding 10 days, and 14 tumours were obtained; *i.e.,* 47 per cent. of the implantations were successful.

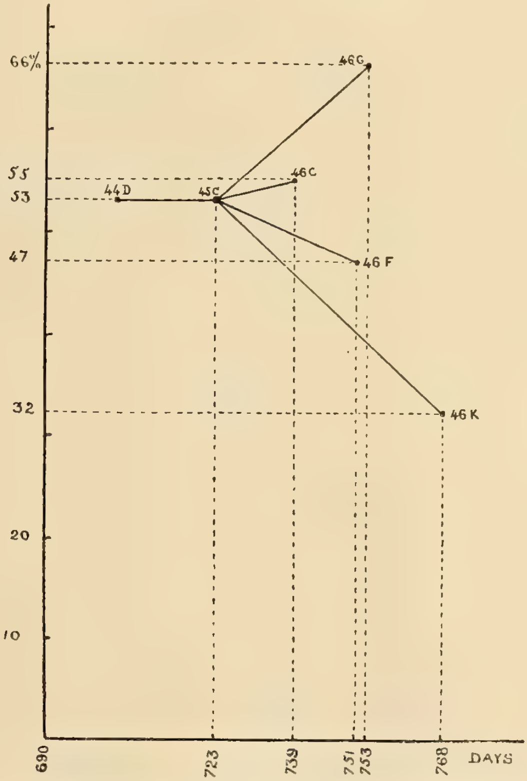

Fig. 1.—Illustrates the method of constructing the graphic records of transplantation experiments (see text).

(3) On the 753rd day of propagation, a third tumour of Transplantation 45, Series C, was transplanted into 40 mice and the experiment labelled 46 G. Eight mice died in the first 10 days following transplantation, and 21 tumours developed in the 32 survivors; *i.e.,* 66 per cent. of the implantations were successful.

(4) On the 768th day of propagation, a fourth tumour of Transplantation 45, Series C, was transplanted into 20 mice, 46 K. One mouse died in the first 10 days and six tumours developed in the remaining 19 mice; *i.e.,* 32 per cent. of the implantations were successful.

The data obtained from these four experiments, viz.:—

	The day of propagation. Abscissa.	The percentage of success. Ordinate.	Name of experiment.
		Per cent.	
1	739	55	46 C.
2	751	47	46 F.
3	753	66	46 G.
4	768	32	46 K.

suffice to determine four points, indicated on the chart by the numbers and letters in the third column.

The four fragments of the *parent* tumour from which the four tumours used for the above experiments (1, 2, 3 and 4) developed were implanted along with 51 other fragments into 55 mice on the 723rd day of propagation, the experiment being labelled 45 C; 23 mice were killed in the first 10 days after transplantation for microscopical examination of the site of implantation; 17 tumours developed in the remaining 32 mice, *i.e.*, 53 per cent. of the implantations were successful. These two numbers, 723 as abscissa marking the date of transplantation, and 53 as ordinate marking the percentage of success of implantations, together fix a fifth point labelled on the chart 45 C. The four points previously obtained represent the results of transplantation experiments on four of these 17 tumours, and to indicate this relation they are each connected with the point labelled 45 C by a straight line.

In the same way a point has been obtained for the parent tumour of 45 C indicated on the chart as 44 D, and similarly for the transplantations antecedent to 44 D and subsequent to 46 G as shown in the larger charts.

The following condensed summary of a number of consecutive experiments will make clear the nature of the results to be recorded in this manner. A tumour of the 39th Transplantation, transplanted into 37 animals, gave tumours in 3 of the 20 animals remaining alive after 10 days (15 per cent.), Transplantation 40, Series I, or shortly 40 I. Of these mice one developed two large tumours weighing together 7·5 grammes in 49 days, when the animal was killed and the tumour transplanted into 24 mice. Tumours developed in 4 of the 20 animals which survived the first 10 days after transplantation (20 per cent.), 41 P; 14 days afterwards one of these tumours weighing 1·3 grammes was transplanted in 66 mice; tumours developed in 7 of the 31 survivors (23 per cent.), 42 L. Of these a tumour, having attained a weight of 4 grammes after 42 days' growth, was transplanted into 45 mice. Tumours developed in 13 of the

43 survivors (30 per cent.), 43 L. One of these, 21 days later, had attained a weight of 3·3 grammes and was transplanted into 27 mice; tumours developed in 8 of the 15 survivors (53 per cent.), 44 D. After 22 days' growth, one of these, 3·7 grammes weight, was transplanted into 55 mice; tumours developed in 17 of the 32 survivors (53 per cent.), 45 C. After 26 days a tumour 1 gramme in weight was transplanted into 40 mice; tumours developed in 21 of the 32 survivors (66 per cent.), 46 G. After 15 days' growth, a tumour weighing 1·3 grammes was transplanted into 40 mice, tumours developing in 26 of the 33 survivors (79 per cent.), 47 H. After 14 days a tumour weighing 1·35 grammes was transplanted into 30 mice, tumours developing in 21 out of 25 survivors (84 per cent.), 48 E. Up to this stage there has been a gradual rise in percentage of success through nine successive transplantations from 15 to 84 per cent. The results of transplanting seven tumours of Series 48 E do not maintain this high percentage. Thus a tumour of nine days' growth, weighing 1·15 grammes, was transplanted into 54 mice, tumours developing in 9 out of 32 survivors (28 per cent.), 49 A. Another, 11 days' growth and 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 out of 38 survivors (33 per cent.), 49 B. A third, also 11 days' growth, weighing 1·4 grammes was transplanted into 31 mice; tumours developed in 10 out of 27 survivors (37 per cent.), 49 C. A fourth, of 15 days' growth, 1·7 grammes in weight, was transplanted into 40 mice; tumours developed in 12 of the 35 survivors (34 per cent.), 49 F. A fifth, of 26 days' growth, 3·2 grammes in weight, was made into an emulsion and injected into six mice. All the mice survived, but no tumours developed, and this experiment is not recorded on the chart below. A sixth, of 27 days' growth, 3·6 grammes in weight, was transplanted into 41 mice and tumours developed in 5 out of 25 survivors (20 per cent.), 49 O. A seventh, after 62 days' growth, weighed 3 grammes. It was transplanted into 40 mice; tumours developed in 20 out of 36 survivors (56 per cent.), 49 X. None of these tumours maintained the high transplantability of the parent growth, although the implantations grew rapidly and were made at intervals of from 9 to 62 days. This sequence in the results has been a constant feature in all the strains propagated, and there is, therefore, reasonable ground for believing that it is a natural feature of growth.

We shall now proceed to a consideration of the graphic records of this series of experiments. In the accompanying chart (fig. 2) the lines joining the points representing the date of transplantation and power of proliferation appear to form a continuous ascending curve rising from 40 I (15 per cent.) through nine successive transplantations to 48 E (84 per cent.).

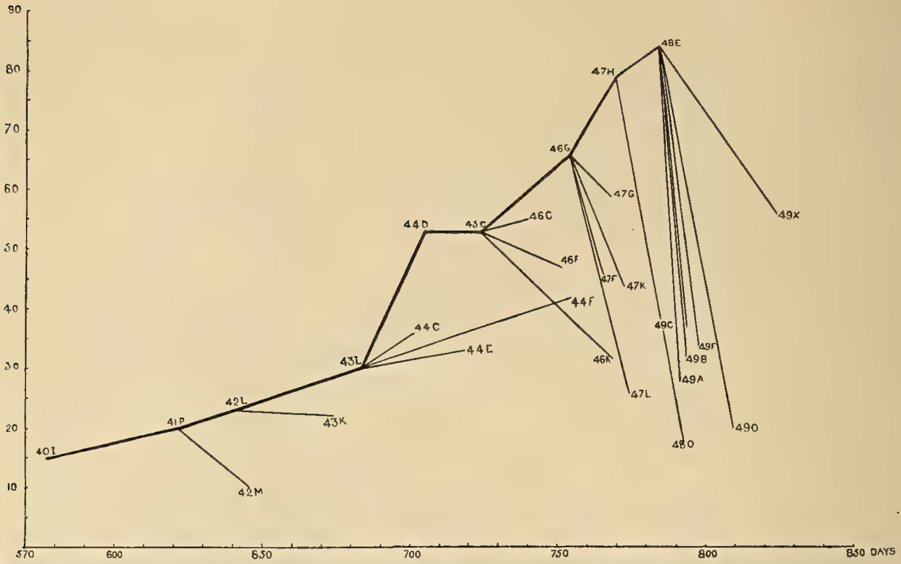

FIG. 2.—Graphic record of steps by which, starting from a tumour giving 15 per cent. of success on transplantation, 40 I, a tumour was obtained after eight subsequent transplantations, giving 84 per cent. of success, 48 E. The success on transplanting some of the other tumours propagated at each step is also recorded by the points at the ends of the lines branching off from the "main stem" of propagation.

When the curve has reached a maximum it falls rapidly in marked contrast to the preceding gradual rise. The seven tumours of 48 E do not all fall to the same level; the degree of the diminution in the success attending subinoculation varies, but the direction of the curve is downwards in all. This sequence of a gradual rise in transplantability followed by a fall has been repeatedly observed during the past two and a-half years. Although in this particular case the fall in percentage of success on transplanting tumours of 48 E is rapid, and attains a minimum at the first essay with many other strains, it has been possible to obtain several estimations on the downward slope of the curve. In such cases, when the tumours of a series following on a maximum are again transplanted, a further diminution in the percentage of success is frequent, if the diminution in transplantability had been slight in the first instance. The accompanying chart (fig. 3) illustrates these points very clearly. It represents the results obtained by transplanting a large proportion of all the tumours of another series, viz., 50 Z, which had indicated 90 per cent. of transplantability. The daughter tumours of this series giving rise to 51 U and 51 T, showed the smallest diminution as compared with 50 Z. From each several tumours have been transplanted, and in each case the diminution in the percentage of success has continued till a level was reached which other tumours fell to at the first essay. The protocol of this experiment is given in full on p. 211.

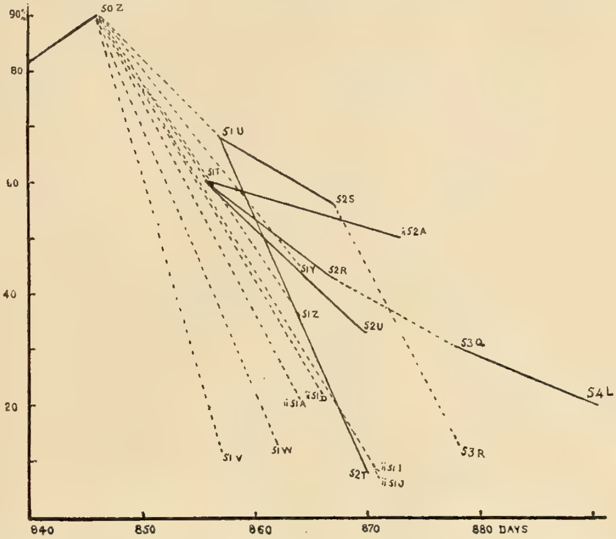

FIG. 3.—Graphic record of further propagation of the majority of the tumours obtained in an experiment (50 Z) in which 90 per cent. of the animals developed tumours. The diminished percentage of success reaches a minimum at the first transplantation in some (51 Y, 51 W); in others after two transplantations (51 U to 52 T and 51 T to 52 U); while in others a third diminution occurs before a minimum is reached (51 U, 52 S, 53 R); and a fourth diminution occurs in the series 51 T, 52 R, 53 Q, 54 L.

The description of the graphic record has so far been confined to illustrating the sequence of events in the tumours in the line giving the highest percentages of success, and their ultimate fate. The other experiments recorded in figs. 1, 2, 4 and 5 must now be considered. The tumours propagated concomitantly with those marking the steps in the ascending curve appear to form the ends of "side-branches" on that curve as a main stem. Some of these "side-branches" also exhibited the upward tendency and duplicated the behaviour of the main stem in that they also rose to a maximum followed by a fall. The others appear to form descending side-branches and to anticipate the ultimate fate of the ascending curve. If the tumours on such descending side-branches be transplanted they may either give a further fall, completely negative results, or gradually increasing percentages of success till they in turn present a maximum followed by a fall.

The most careful attempts to maintain the percentage of success at a high level in the direct line of descent therefore show that the condition leading to diminished transplantability ultimately affects the descendants of the tumours which had previously escaped it, and hence appeared to constitute an ascending main stem in the graphic record. We have always obtained a rise to a maximum which cannot be maintained, and a subsequent fall which is also not permanent if continued propagation be possible. Up to the present we have encountered no exception to this rule in more than 600 series of inoculations with this tumour, and the rise to a maximum with a subsequent fall has been repeated 50 times in simultaneous series of experiments. If the subsequent behaviour of the descendants of several of the daughter tumours from any one batch of inoculations be followed, successive maxima are seen to arise one after another at short time-intervals. The maximum percentage of success of the experiments as a whole is maintained continuously at a high level between 70 and 90. Each strain, after reaching its maximum, falls and makes way for another which had previously presented a lower percentage, and, after attaining a maximum, in turn falls. A high percentage is thus maintained by successive maxima developing in parallel series of experiments.

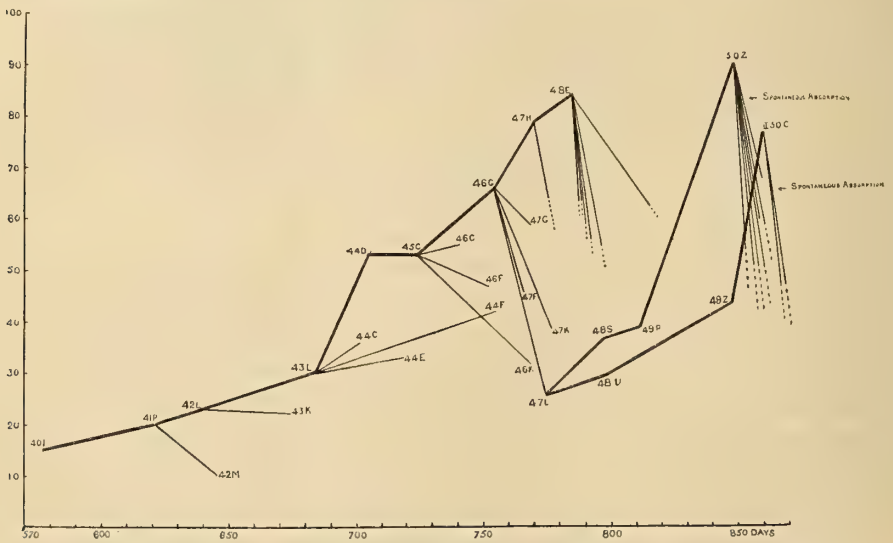

Fig. 4.—Graphic record of results of further propagation of two tumours of an experiment in which 32 per cent. of the animals developed tumours (47 L). Both of these gave an increasingly higher percentage of success till a maximum (50 Z and ii 50 C) followed by a fall was again obtained. The dotted lines are not completed to the point at which they should end, but merely indicate the downward direction of the curve. The details of the fall following the maximum 50 Z are given in the preceding graphic record fig. 3.

The preceding diagram shows clearly the manner in which successive maxima develop. The subsequent behaviour of two tumours of one of the "descending side-branches" of the "rising main stem" depicted in the earlier chart (fig. 3) is followed through four successive subinoculations till each strain in turn presents a maximum followed by a fall. The same phenomenon is repeated in the experiments recorded in the next chart (fig. 5), where the further results of transplanting two strains derived from 48 E are represented. After giving a low percentage of success, both in turn give a maximum.

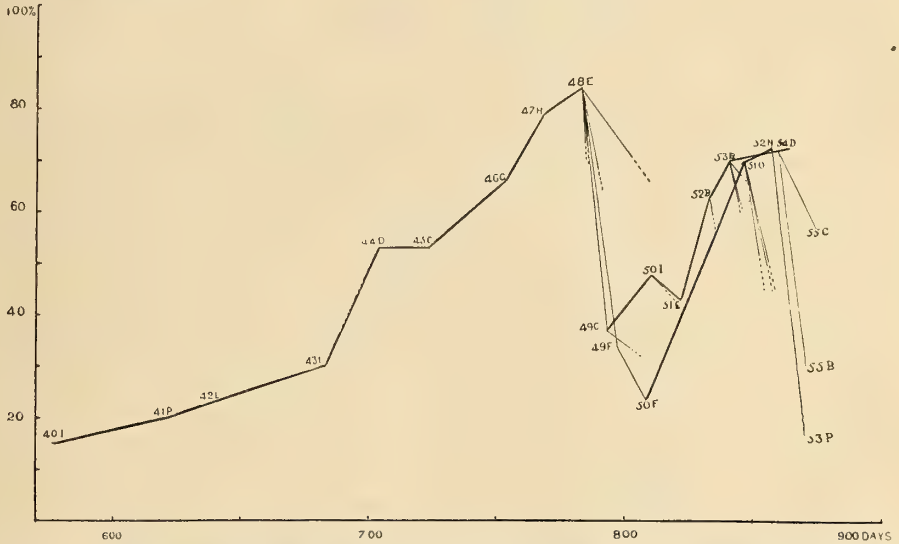

Fig. 5.—Graphic record to show how the further propagation of two tumours (49 C, 49 F) obtained in an experiment with maximal percentage of success (84 per cent. 48 E) gives at first a diminished percentage of success, which, after a varying number of transplantations (in one case six, in the other three), is succeeded by a maximal success after the same interval of time after which the fall is repeated.

When the results of all our experiments are incorporated in one chart it becomes very complex. The orderly sequence of increasing and diminishing percentage of success in individual strains can be followed with difficulty. The confusion, however, is only apparent and indicates how heterogeneous the growth of the tumours viewed as a whole has become. The behaviour of the component parts of this tumour when propagated in a large number of animals represents what may be regarded as occurring simultaneously in different parts of a single tumour, when allowed to grow for a long time in one animal. During the whole course of propagation of Jensen's tumour, after each successful transplantation, the differences in transplantability of the daughter tumours indicate that heterogeneity of this kind develops. Only when, after a number of passages, a tumour is obtained giving the maximum percentage of success is there any approach to homogeneity in percentage of success on transplantation. After a time any single tumour cannot be regarded as consisting of cells of equal proliferative power. Just as a composite chart of all the strains propagated indicates their very different behaviour at any one date, so in any single tumour at one part growth is proceeding actively, at another growth is proceeding slowly or actually ceases. The same heterogeneity may be postulated for sporadic tumours. In all probability sporadic tumours owe their apparently continuous growth to the simultaneous presence in different areas of numerous growing centres. These mask the effects of concomitant degeneration, and account for the rarity of spontaneous absorption among sporadic as compared with transplanted tumours. The greater frequency of cessation of growth followed by spontaneous absorption in experimental tumours seems to be due to the greater homogeneity resulting from the limited number of centres of growth represented in any one implantation.

The spontaneous absorption of the whole of a transplanted tumour is rare. In the living animal it is preceded by cessation of growth. The tumour apparently remains of the same size for a period of one or two weeks. It gradually diminishes in size, and if examined histologically at this stage, the parenchyma is found to be broken up into small masses and often surrounded by a zone of large phagocytes, external to which there is an overgrowth of sclerosing connective tissue. The process is indistinguishable from what is frequently observed in circumscribed areas in large tumours, and from that which we have described with Dr. W. Cramer* as occurring when tumours disappear under the action of radium. In large tumours in which growth, the cessation of growth and the tendency to absorption show themselves side by side, large cysts are often encountered filled with serum slightly stained with blood. The relation of spontaneous absorption to a definite phase in the fluctuations in transplantability is in our experience a very close one. It occurs most frequently when a high percentage of success has been obtained, and coincides with the time when rapidly growing tumours show a great diminution in the percentage of success on transplantation.

This association with a definite phase in the fluctuations has already been indicated for two strains on chart fig. 4. It is additional evidence that the diminished transplantability is due to a real alteration in the parenchyma cells, inability to establish themselves in new animals coinciding with the spontaneous cessation of growth in an animal in which growth had already been established. The following experiment illustrates this association in the clearest manner:—

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* 'Second Scientific Report of Imperial Cancer Research Fund,' Part II, pp. 59—60.

Transplantation 50, Series iiC.*

Parent tumour. Chocolate and white coloured young male of Transplantation 49, Series Z. Attained a weight 0·65 gramme in 12 days. Very soft consistence. No necrosis. Transplantation was effected into:—

(1) 7 mice 5 days old.
(2) 6 „ 4 „
(3) 10 „ 10 „

(3) were all dead within 10 days. In the remaining 13 mice 10 tumours were evident after 10 days and grew rapidly (77 per cent.). Their subsequent history is as follows:—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplantation.	Result of microscopical examination.
		grammes.	grammes.			
1	10	5	0·3	No necrosis ……………	51, iiH. No tumours in 10 mice.	Early stages in spontaneous absorption.
2	16	—	Had diminished in size.	… …	Not transplanted. Preserved entire.	Spontaneous absorption in progress.
3	16	6·22	0·28. Had diminished in size.	Two tumours, both necrotic. Anterior tumour firm and yellowish as if undergoing absorption.	51, iiK. 1 tumour in 9 mice. 11 per cent.	Do.
4	29	7·2	4·12	Diffuse necrosis almost complete, thin layer of healthy tumour immediately subjacent to skin.	51, iiM. 10 tumours in 16 mice. 63 per cent.	
5	31	5·95	3·15	Complete necrosis. Thin layer of healthy tumour on deep surface.	51, iiN. 12 tumours in 19 mice. 63 per cent.	

The tumours, at first growing rapidly, in the other five mice ceased growing two weeks after inoculation, and, after remaining stationary for a few days, diminished rapidly in size, and had disappeared entirely 21 days after inoculation. The five animals and the three which did not develop tumours were then re-inoculated.

The tumours obtained in this experiment form a graduated series. In the first tumour (1), inoculated after 10 days' growth, the results were completely negative, no tumours developing. A tumour (2) was preserved entire after 10 days' growth, and showed the histological features of spontaneous absorption, while another (3) transplanted on the same day with the same histological appearance gave 11 per cent. of success. Five tumours (6 to 10) of large size which were not interfered with disappeared spontaneously. Two tumours (4 and 5) continued to increase in size, in each case attaining half the weight of the mouse in which they were growing. They were almost entirely necrotic, but the healthy portions on being transplanted gave 63 per cent. of tumours in each case, both had apparently recovered from the negative phase fatal to those spontaneously absorbed and causing a negative result, or low percentage of success in those transplanted after 10 and 16 days' growth respectively. This one experiment presents all the phenomena, usually only revealed by a study of several consecutive series. The protocol should be compared with that of experiment 50 Z, given on p. 211. The close genealogical relationship of these two experiments so strongly corroborative of each other is shown in the chart, fig. 4, p. 206. If followed backwards, both are seen to arise from tumours of series 47 L, a descending "side-branch" on the "ascending stem" described on an earlier page.

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* When the series in one transplantation have arrived at the letter Z, we commence again at A and prefix the numeral "ii."

From a review of the observations recorded in the preceding pages we conclude that the proliferation is only apparently continuous. In reality it is made up of a succession of alternating phases of increased and diminished energy of growth.

In the preceding pages we have concerned ourselves solely with estimates of the power of proliferation throughout a long time, although the extent to which cell degeneration goes hand in hand with cell proliferation is remarkable (see two following protocols and table of experiments on pp. 216 and 217). Growth is always accompanied by extensive degeneration of the cells of the tumours. The histological examination of all the tumours propagated has been systematically performed, and shows that, just as all the cells are not equally capable of continuing growth, so all are not histologically identical in any tumour. The histological difference most easily observed is that rapid and complete degeneration which attacks the central areas of the alveoli in which the parenchyma cells are arranged.

Transplantation 50, Series Z.

Parent tumour. Young brown female of Generation 49, Series F. Tumour weighed 5·65 grammes. Mouse alone weighed 9·3 grammes. Two tumours in medial line of back. Anterior practically completely necrotic with thin healthy layer on deep surface adjacent to muscles. Posterior tumour practically complete necrosis with thin healthy layer under skin. Skin slight early ulceration. The posterior tumour penetrated abdominal wall, pressing on and displacing kidney.

Transplantation into 40 young normal mice, of which two died within 10 days. In the remainder 32 tumours developed as under :—

No. of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.	Result of transplanting.
		grammes.	grammes.		
1	11	10·7	1·45	No necrosis	60 per cent. 51 T.
2	12	10·3	1·3	Early diffuse necrosis ...	68 „ 51 U.
3	12	9·7	0·85	„ „ ...	12 „ 51 V.
4	14	10·0	Too small to weigh.		
5	17	14·8	1·82	Diffuse marked necrosis	13 „ 51 W.
6	18	9·8	1·1	Central necrosis.	
7	19	8·9	0·2	No necrosis.	
8	19	17·5	1·34	Early diffuse necrosis ...	45 „ 51 Y.
9	19	8·45	1·4	„ „ ...	35 „ 51 Z.
10	19	7·66	1·84	„ „ ...	21 „ 51 iiA.
11	20	11·9	0·9	Complete necrosis.	
12	20	13·2	0·3	Central necrosis.	
13	20	9·1	2·4	Necrosis almost complete, therefore difficulty in transplanting	53 „ 51 iiB.
14	20	9·05	0·95	Marked necrosis.	
15	20	19·65	1·05	Marked diffuse necrosis	22 „ 51 iiD.
16	21	10·7	1·1	Complete necrosis ulcerated.	
17	21	9·9	0·7	Diffuse necrosis.	
18	21	15·05	0·55	Practically complete necrosis.	
19	21	8·2	0·9	Early very slight necrosis	28 „ 51 iiF.
20	21	11·35	1·15	Early slight necrosis......	25 „ 51 iiG.
21	23	10·3	1·3	Marked necrosis.	
22	25	10·1	0·5	Diffuse necrosis.	
23	25	9·0	1·0	„	7 „ 51 iiJ.
24	25	12·8	0·6	Very slight early necrosis	8 „ 51 iiI.

25 to 32.—Nine other tumours ceased growing after three weeks and were ultimately completely absorbed. The mice were then re-inoculated.

Transplantation 51, Series T.

Parent tumour. Young white female of Transplantation 50, Series Z. Tumour weighed 1·45 grammes, mouse alone weighed 10·7. Tumour situated between scapulæ, soft in consistence, very vascular, no hæmorrhage, no necrosis.

Transplanted into 40 normal young mice, of which 10 died within 10 days; in the remainder 18 tumours developed as under:—

Number of mouse.	Day of growth.	Weight of mouse.	Weight of tumour.	Naked-eye appearance.
		grammes.	grammes.	
1	10	11·8	0·3	No necrosis.
2	11	14·35	0·9	"
3	14	12·75	0·8	Slight diffuse necrosis.
4	14	8·3	1·1	Very slight necrosis.
5	15	9·2	1·3	Diffuse necrosis.
6	16	6·95	1·16	No necrosis.
7	17	14·6	0·65	Central necrosis.
8	17	12·2	0·2	Slight diffuse necrosis.
9	17	11·85	2·15	Diffuse necrosis.
10	17	16·7	1·0	No necrosis.
11	17	14·8	0·7	Diffuse necrosis.
12	17	13·6	2·25	"
13	17	13·7	0·8	Slight central necrosis.
14	17	14·2	1·85	Diffuse necrosis.
15	17	8·4	0·9	Marked necrosis.
16	17	8·45	Too small to weigh.	
17	17	15·4	"	
18	17	13·6	"	

Of course cells presenting complete degeneration are no longer capable of giving rise to tumours. In fact they are rapidly taken up by phagocytes in the days immediately succeeding transplantation, and it might be concluded that growth was continued by cells which never even tended to degenerate. Parts of the tumours which do not present this central necrosis are not of uniform histological structure. Fig. 6 presents a histological appearance common in these tumours (when preserved in strong Flemming solution) in the portions apparently healthy to the naked eye. Dark and clear areas are seen, the darkly stained portions which usually border on the connective tissue being due to a progressive degenerative process in the cells.

The cells which present this condition in any marked degree degenerate immediately after transplantation, while growth is mainly continued by the clear cells, and it is interesting to note that such degenerating cells form a large proportion of tumours exhibiting early phases in spontaneous absorption.

The effects of eliminating degenerating cells at each transplantation for the series 40 I to 48 E (*vide* p. 202) can be indicated by employing the percentage of success to construct a diagram of the relative proportions of implanted fragments which developed into tumours or were absorbed respectively. The percentage of success in a batch of inoculations is a test of the constitution of the parent tumour. If a series of large squares represent the series of experiments giving a maximum at 48 E, the constitution of the parent tumours as revealed by the percentage of fragments developing into tumours can be depicted by subdividing each large square into 100 small squares each representing an implanted fragment, and blackening as many as there were implantations which did not yield tumours. In the accompanying diagram (fig. 7) the clear part of each large square represents the percentage of success attending the transplantation of a tumour arising from a single small square in the one before it. We may imagine that the blackened part of each square (fig. 7) represents those implanted fragments of tissue which, healthy at the time of inoculation, are already on the way to degeneration and do so degenerate immediately after transplantation. The continuous diminution in successive subinoculations which this blackened part undergoes, as the number of fragments developing into tumours

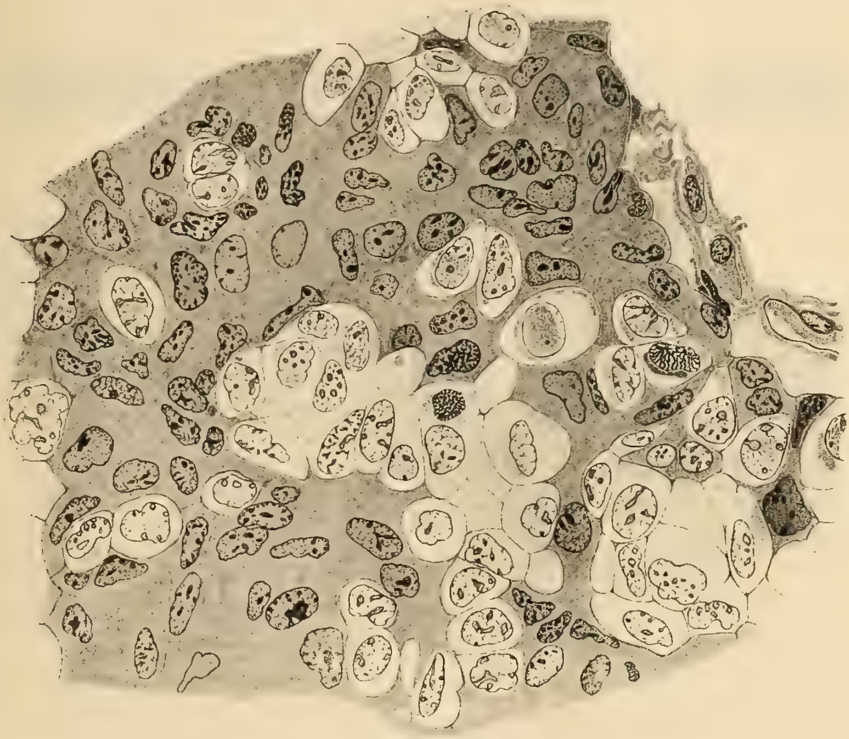

Fig. 6.—Histological differences between cells in a tumour apparently healthy and homogeneous to the naked eye. Islands of clear cells, whose nucleus and protoplasm have little affinity for stains, are surrounded by cells whose nucleus and protoplasm stain intensely. The latter are more numerous on the surface of the tumour alveoli. This differentiation is very frequent in tumours undergoing spontaneous absorption.

increases, would then merely indicate the elimination of degenerating tumour cells by the selection exercised at transplantation and the further elimination occurring in the days immediately following. Tumours are ultimately obtained free from the original admixture of such doomed cells. They consist entirely of the progeny of those healthy cells (in the first tumour of the series) which were destined to carry on growth. Even the progeny of those healthy cells ultimately enters upon a degenerative phase, as is shown by the sudden reappearance in the diagram (49 C) of a large blackened area when the clear area has attained a maximum. The increased tendency to degeneration reappears over a considerable interval, as a further reduction of the clear area in the diagram at 50 L and 51 L indicates. Thus the tendency to degenerative changes is intercalated in the course of the continued proliferation of the parenchyma cells.

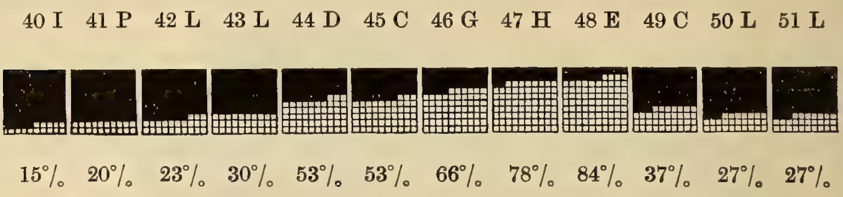

Fig. 7.—Diagram to illustrate the way in which the elimination of degenerating cells by repeated transplantation may result in a progressive increase in the percentage of success in a strain of transplantations. Each large square represents the constitution of the parent tumour of the batch of inoculations whose label is printed above it, as measured by the percentage of success printed below. One hundred inoculations are supposed to be made in every case, and the number of small squares left clear, corresponding to the percentage, shows the number of fragments which developed into tumours.

Our methods of propagation and of recording the results enable us to analyse the growth of small groups of cells. So far as the descriptions of experiments published permit us to form an opinion, other investigators have emulsified single tumours, or have emulsified and mixed several tumours, and injected portions of the emulsion. This method maintains a mixture of strains at each inoculation and they have therefore recorded the results as *average* percentages of all subinoculations made after the same number of transplantations, no detailed analysis of the features of growth being attempted. Other authors, therefore, do not give the details of the behaviour of single strains, and we are unable to compare their results with our own. The increase in the percentage of success in our later transplantations as compared with the earlier ones obtained by Jensen himself must not be confounded with a permanent alteration in the character of the cells as the result of the number of transferences from animal to animal. The highest percentage of success obtained by Jensen* is given as 66 per cent. for single experiments. We recorded in March, 1904,† success in 90 per cent. of the animals used at the third transplantation into English mice. Since then we have repeatedly obtained from 80 per cent. to 100 per cent. of success in individual strains in the manner already described. The variations in percentage of success appear to be quite irregular when recorded in tables giving either the average percentage of success for the successive series of transplantations, or the results of individual experiments in each transplantation (see Table on pp. 216 and 217). The confusion presented led us to study the percentage of success in greater detail in single strains, with the result that the irregularities resolved themselves into the orderly sequences we have described.

The experiments we have already described, and the graphic records pertaining to them, have enabled us to follow the behaviour of single strains in the direct line of descent. The phase of growth brought out by maximal success on transplantation is the same in separate strains if the fluctuations have any meaning at all. In the same way the minimal success represents the opposite phase of growth. In the accompanying graphic record (fig. 8) the minimum is reached after one transplantation in Experiments 51 V. 51 W, 51 iiA, 51 iiI, and 51 iiJ; after two in Experiments 52 T and 52 U;

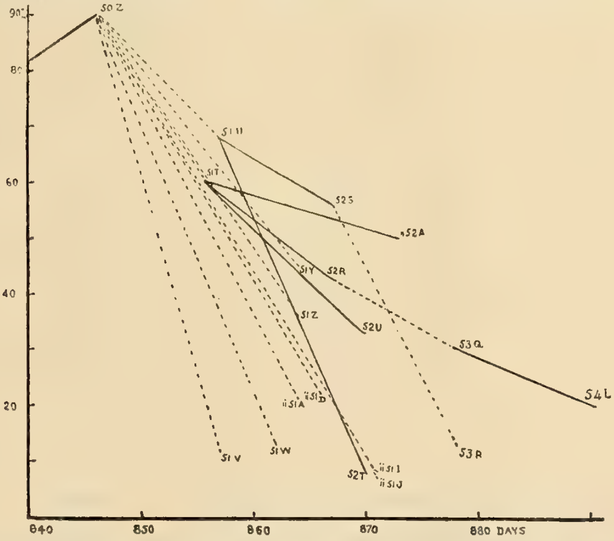

Fig. 8.—Graphic record to show that the same stage of proliferative activity is not always reached after the same number of transplantations. (Repetition of fig. 3.)

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* 'Centralblatt f. Bakt.,' vol. 34, 1903.

† 'First Scientific Report of Imperial Cancer Research Fund,' p. 14. *Cf.* also 'Second Scientific Report,' pp. 22 and 54.

after three in Experiment 53 R; and after four in Experiment 54 L. Thus the number of successive transplantations while furnishing a convenient label for experiments does not indicate corresponding stages in all the experiments. The number of times a tumour has been successively transplanted from animal to animal does not give any indication of what its future behaviour on transplantation is likely to be. That is determined mainly, if not entirely, by its previous behaviour as recorded in the curves, although we are not yet able to predict the immediate results of transplantation in any one case with certainty. In every transplantation series of implantations are obtained with maximal and minimal percentages of success. Series are obtained in later transplantations in which, on a large number of animals, percentages occur as low as in the earlier transplantations and occasionally negative results. They show clearly that the transplantation to which a tumour belongs does not of itself determine its

Transplantation.	Average percentage in each transplantation.	Percentage of success													
		A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.	N.
23															
24	5														
25	14·3	15	30	21	7	4	9								
26	22·9	5	16	**8**	—	10	2	**41**	**19**	**36**	**47**	**18**	**12**	**25**	24
27	25·2	**29**	37	**90**	**17**	**14**	**29**	**25**	16	**9**	—	**17**	—	**43**	**16**
28	15·8	**11**	**10**	—	**40**	20	—	**16**	—	—	**10**	4	—	8	—
29	12·8	13	6	**18**	—	—	5	**15**	**12**	—	**20**	**10**	**17**		
30	15·6	**13**	**10**	**13**	16	**10**	—	**18**	—	**21**	**33**	**7**	—		
31	29·5	**5**	**92**	**23**	**10**	**37**	**30**	**25**	**50**	**50**	**13**	—	**11**	**13**	—
32	29·6	**28**	**44**	**40**	—	15	**67**	—	—	**4**	**7**	—	**32**	—	
33	17·5	25	**11**	**4**	**25**	**25**	**17**	**9**	8	—	**20**	—	**38**	4	—
34	14·6	**15**	**15**	**9**	—	**10**	**8**	—	**7**	—	**15**	5	**4**	**16**	**27**
35	19·8	**33**	10	**17**	**10**	10	—	—	—	22	14	20	**24**	20	31
36	23·5	37	—	—	**24**	**32**	10	**13**	30	**25**	40	40	—	31	**13**
37	24·0	**19**	**8**	**11**	21	—	—	32	31	**28**	**10**	30	45	25	**33**
38	30·0	25	—	**18**	25	100	75	**33**	—	—	25	—	—	17	20
39	25·8	50	29	—	—	13	**33**	**17**	9	—	—	**14**	—	—	**11**
40	28·7	63	31	33	22	—	—	—	**13**	**15**	**33**	**21**	12	—	—
41	24·8	31	29	36	**14**	17	**33**	**13**	**17**	21	—	**38**	**11**	**17**	**5**
42	21·7	—	44	29	25	—	**9**	—	**14**	—	—	—	23	**10**	**11**
43	17·8	—	—	16	20	22	**4**	—	5	—	—	**22**	**30**	—	**33**
44	38·8	27	**44**	**36**	**53**	**33**	**42**	63	13	—	—	—	—	—	—
45	32·2	29	**33**	**53**	**13**	33	—	—	—						
46	41·2	—	—	55	—	—	47	66	**46**	**55**	—	**32**	16	33	
47	48·9	23	—	72	47	—	46	59	79	75	**61**	**44**	**26**	62	**73**
48	43·7	—	30	43	**83**	84	—	47	**70**	**50**	—	60	**50**	**50**	**57**
49	40·5	28	33	37	**3**	33	**34**	50	50	29	—	**43**	**33**	—	39
50	41·1	58	**54**	71	55	—	24	**61**	**30**	48	—	—	30	14	40
51	37·5	78	—	7	60	43	43	**64**	**66**	48	40	**18**	**27**	**21**	32
52	36·2	36	63	**66**	74	38	6	33	**10**	7	**5**	**48**	**45**	**22**	**72**
53	39·6	43	70	40	26	48	—	43	38	44	53	—	**56**	**54**	**38**
54	32·2	13	—	64	72	6	11	47	47	60	—	—	20	8	6

The figures in blacker type refer to tumours which behaviour. They are as clean experiments as those with high percentage, and must be included in an objective consideration of the energy of growth of the tumour as measured by percentage of success. Recording the results by the average percentage of all the series in each transplantation therefore obscures the behaviour of individual strains and fails to reveal the composite nature of this apparently continuous proliferation.

in each transplantation.

O.	P.	Q.	R.	S.	T.	U.	V.	W.	X.	Y.	Z.	Aii.	Bii.	Cii.	Dii.	Eii.	Fii.	Gii.	Hii.	Iii.	Jii.
—	—	21	33	—	—	50															
40	18	5	28	30	20	14	7	25	25	33											
17	—	9	20	—	13	14	27	—	—	5	30										
—	25																				
—	9	—	—	33																	
3	17	7	—	—	5	52	25	24													
44	7	35	6	31	16	—	8														
32	19	8	8	14	—	35	13														
9	—	67	2	—	33	15	30	—	8												
19	37	10	14	30	24	47	—	—	—	—	33	18	33	—	—	18	33	8	—	28	
21	9	—	33	27	67	—	—	10	—	—	43	27									
40	42	4	50	18	29	15	38														
20	20	36	35	59	40	25	13	15	9	26	41										
39	18	13	9	3	—	—	—	59	20	—	6										
15	6	24																			
13																					
69	61	—	33	34	6	7	46	65													
18	—	50	—	37	67	30	32	22	36	10	71	18	12	23							
20	39	—	63	73	40	74	36	37	56	39	44										
—	15	37	57	6	59	59	15	15	37	53	90	40	14	77	19	31					
70	29	64	—	—	60	68	12	13	19	45	35	21	53	—	22	—	28	25	—	8	7
32	—	33	43	56	8	33	14	80	25	—	12	50	57	42	31	40	—	20	53	7	
47	16	30	13	11	19	63	66	—	—	10	43	8	—	—	—	59	44	80	—	33	15
—	—	—	40	—	—	25															

were necrotic on naked-eye examination.

In the above table the average percentage for each transplantation and the percentage of success for each series are tabulated in numerical and in alphabetical order. Since high percentages were obtained in early transplantations, the apparently progessive increase in the percentage of success in later ones cannot be regarded as indicating a permanent alteration in the powers of growth of the parenchyma cells as a result of the number of passages from animal to animal. The error of such an interpretation is demonstrated by a consideration of the accompanying chart (fig. 9), which

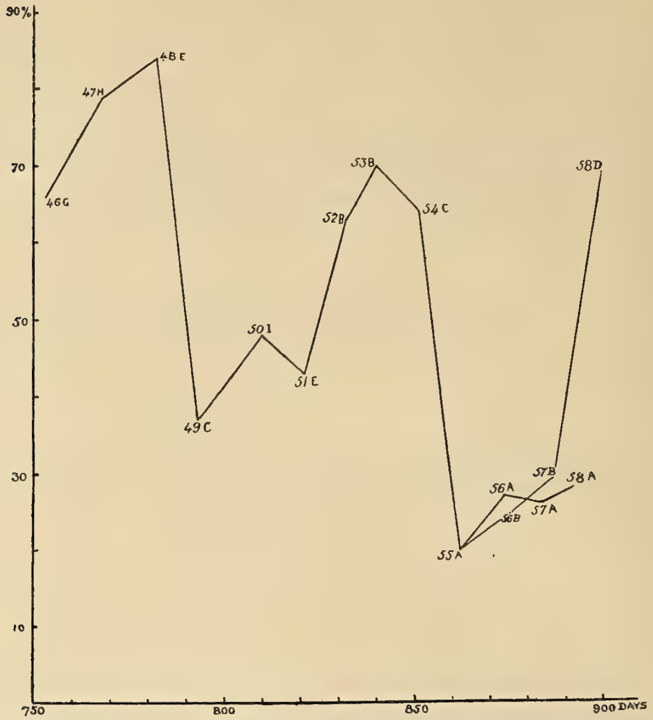

Fig. 9.—Graphic record of a strain of 13 successive transplantations at short intervals The fluctuations already described appear in this series also.

gives the results of rapid passage from animal to animal from the 46th to the 58th transplantations. This series of experiments was rendered possible by the rapid growth of certain tumours arising at each subinoculation. So far from resulting in a progressive increase in transplantability, the 55th transplantation presents the lowest percentage in the whole series. The fluctuations already described for other strains are present here also, when the interval between successive subinoculations is shortened to intervals of 8 to 15 days. Such a curve, representing a succession of rapid transplantations, is a special case like those depicted in the curves* we have already published.

In the preceding pages it is assumed (1) that the conditions have been sufficiently uniform throughout the experiments to exclude fortuitous fluctuations, and (2) that the percentage of success on transplantation furnishes a reliable measure of power of proliferation. If the precautions we have taken warrant these two assumptions, we are entitled to conclude that the fluctuations in proliferative power revealed are natural features of the growth of Jensen's tumour in English mice. They are due to the acquisition of renewed powers of growth by the cells when proliferation is becoming exhausted and may actually terminate, resulting in the spontaneous absorption of tumours which had established themselves and grown for a time.

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* 'Second Scientific Report of Imperial Cancer Research Fund,' Part II, p. 54.

From time to time sporadic mammary tumours have occurred in the mice purchased for these investigations, and with all artificial propagation has been attempted. The resulting proliferation has in no case been equal to that obtained with Jensen's tumour. Thus out of 20 sporadic tumours transplanted the primary implantations have been negative in 9. We select for detailed description the features of the proliferation resulting from the propagation of two sporadic tumours (namely, VII and XIX) which exemplify the behaviour of tumours capable of only limited propagation. Both could be transplanted several times from animal to animal. The first was transplanted into 133 mice. One tumour developed in the 12 mice remaining alive after 10 days. It was transplanted after 20 days' growth into 24 mice. One tumour developed in the four mice which remained alive after 10 days. It grew slowly, attaining a diameter of 2 cm. after 184 days' growth, when it in turn was transplanted into 208 mice. Two tumours developed in the 80 mice which survived the first 10 days. They were transplanted after 48 and 66 days' growth respectively. No tumours developed in either case, and the experiment came to an end. The accompanying graphic record (fig. 10) shows the contrast which obtains between the artificial propagation of such a tumour and that of Jensen.

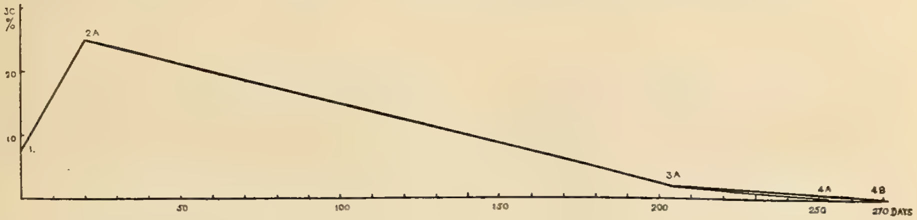

FIG. 10.—Graphic record of propagation of a sporadic mouse tumour VII. Shows a temporary rise (?) in transplantability and extinction of the tumour at the fourth transplantation.

Another sporadic mammary tumour (XIX) was removed by operation and transplanted in 85 mice. Fifteen tumours developed in 75 mice remaining alive after 10 days. The accompanying graphic record (fig. 11) shows the results of the transplantations.

The ultimate fate of the propagated tumours in these two cases was the same. The interest of the second case lies in the fact that the sporadic tumour recurred rapidly after operation, which was rendered necessary on three subsequent occasions. The tissue obtained at these operations was transplanted under the same experimental conditions. From the implantations after the first operation fifteen tumours developed in the 75 mice remaining alive 10 days after implantation. Nine of these tumours were used for transplantation. The other six grew for a time, attaining an approximate weight of 0·5 gramme, then remained stationary, and were ultimately completely absorbed. The histological features of spontaneous absorption were identical with those already alluded to. One tumour developed in the 50 mice alive 10 days after inoculation with the material from the second operation. Negative results were obtained with material from the other two operations, 81 implantations having been made in each instance. When the mouse died three months after the first operation the left pleura and right lung were found filled with metastatic deposits. Thus growth proceeded in the animal primarily affected, and at the same time ceased in the animals inoculated succesfully, either after a transitory proliferation in one animal or in succeeding transplantations. Portions of the growth removed at subsequent operations did not exhibit the same proliferative energy, when transplanted, as those obtained at the first operation. We wish to draw attention to the similarity of behaviour of this sporadic growth at different times with the behaviour of single strains of Jensen's tumour in which cessation of growth

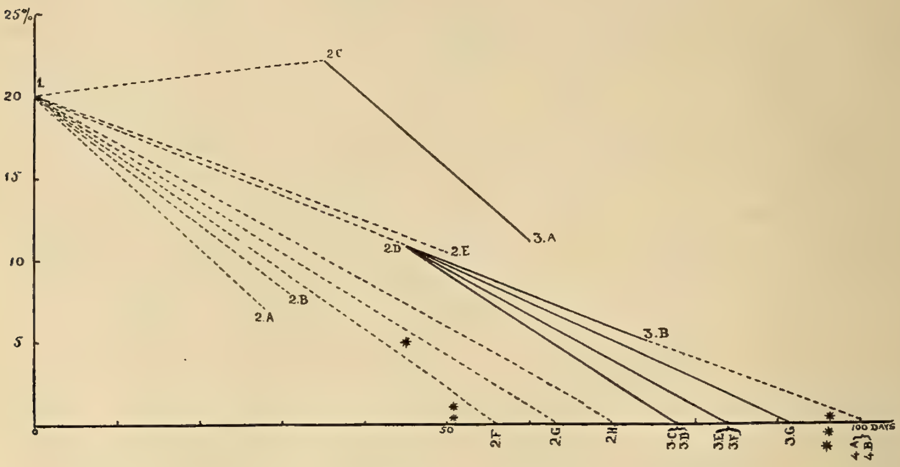

FIG. 11.—Graphic record of propagation of a sporadic mouse tumour XIX. Commencing at 20 per cent. the transplantability gradually diminished through three successive transplantations till negative results were obtained. The tumour was removed by operation in the first instance, and recurred three times. The results of transplanting the material obtained at these three later operations are indicated also on the chart at * 5 per cent., ** 0 per cent., and *** 0 per cent.

(*cf.* fig. 12) and spontaneous absorption (*cf.* figs. 3 and 4) supervene on a high degree of transplantability and when negative results are obtained either immediately, or by graduated steps, when a tumour of a series giving a high percentage is transplanted.

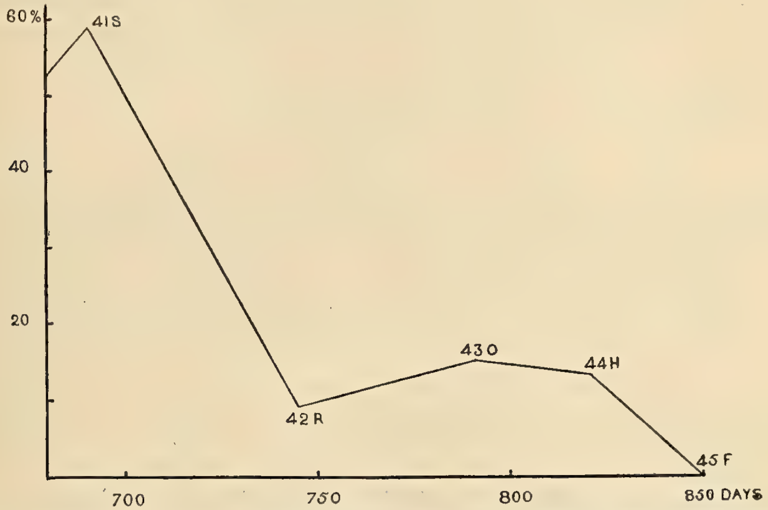

Fig. 12.—Graphic record of propagation of a strain of Jensen's tumour which gradually gave a lower and lower percentage of success till a negative result terminated growth. *Cf.* fig. 10 and fig. 11.

Many tumours of the mouse's mamma give negative results on transplantation, and in this respect resemble the tumours of the other longer-lived mammals. Of those, in which the primary transplantation is successful, the later results often show a gradually diminishing percentage of success till, finally, negative results are obtained, on transplantation. The enormous proliferation obtained with Jensen's tumour is exceptional. Growths, undoubtedly malignant, are not necessarily equally capable of artificial propagation. As our experience of malignant new growths in mice widens, the power which small fragments of tumour possess of establishing themselves in new hosts on successful implantation is found to be rarer than might be expected from the frequency of metastasis formation, to which it is closely related. The factor or the factors actively responsible for the development, the continued growth, and the formation of metastases of the different sporadic tumours in the animals primarily affected are not equally efficacious in ensuring a continuance of proliferation under the similar experimental conditions of artificial propagation. We must therefore conclude that the causative factors have operated with varying intensity, or that additional factors are superadded in some cases.

The behaviour of some strains of Jensen's tumour present a parallel to the other tumours now under consideration. We have already alluded to the negative results sometimes obtained on transplanting Jensen's tumour. In the graphic record (fig. 12) the steps are shown by which a strain of Jensen's tumour, at first giving a high percentage of success, progressively exhibits weaker and weaker powers of proliferation, till finally the tumours obtained gave negative results on transplantation. Such strains are not uncommon; they have frequently been followed to a finish during our experiments. Thus one chapter, as it were, in the life history of Jensen's tumour reproduces the entire life history of other tumours under artificial propagation. These results are difficult to harmonise with the assumption that the apparently continuous proliferation of Jensen's tumour is purely vegetative. Together with the facts of spontaneous absorption they strengthen the conclusion derived from a study of the details of that proliferation, that a cyclical process is involved.

The importance of the preceding analysis of the growth of propagated cancer is obvious in appraising the results of attempts to modify growth experimentally. The experimental conditions whose variations cause irregularities in the success of artificial propagation must be taken account of. In particular, the age of the animals would seem to call for especial attention, because the short duration of the life of a mouse magnifies the effect of the lapse of time involved in procedures for inducing immunity. Specially adapted control experiments must be performed in order to obviate the fallacy which the ageing of the animals introduces. Those fluctuations which cannot be referred to the experimental conditions but are natural features of the proliferation of the tumour cells are an even more urgent reason for caution in interpreting the results of therapeutical experiments. The difficulty or even impossssibility of predicting the time at which spontaneous absorption will affect the propagated tumours indicates the necessity for accurate records of their previous history.

In another paper with Dr. Cramer we shall discuss the results we have obtained on re-inoculating mice in which the absorption of well-established tumours had occurred spontaneously and under the action of radium.

Sex-determination in Hydatina, *with Some Remarks on Parthenogenesis.*

By R. C. Punnett, Fellow of Gonville and Caius College and Balfour Student in the University of Cambridge.

(Communicated by Adam Sedgwick, F.R.S. Received May 15,—Read June 28, 1906.)

[Plate 11.]

Introductory.—Those writers who hold that the sex of an animal organism can be affected by a modification of external conditions during the period of development have of late years supported their case by reference to the parthenogenetic rotifer, *Hydatina senta.* Upon certain of the facts of reproduction there is a general consensus of opinion among those who have made a study of this species. Briefly summarised it is as follows:—

Three kinds of ova are produced by this rotifer, viz.: (1) parthenogenetic ova which develop into ♀'s; (2) parthenogenetic ova of smaller size which develop into ♂'s; and (3) fertilised eggs which always develop into ♀'s. Any given ♀ lays but one of these three classes of eggs during her lifetime. We may, therefore, distinguish three kinds of ♀'s by the eggs they lay, viz.: (*a*) ♀'s which produce ♀'s parthenogenetically, or *thelytokous* ♀'s; (*b*) ♀'s which produce ♂'s parthenogenetically, or *arrenotokous* ♀'s; and (*c*) the layers of fertilised eggs. Both thelytokous and arrenotokous ♀'s can be impregnated by the ♂. On the former impregnation has no effect. In the case of the arrenotokous ♀'s impregnation has no effect, unless it occurs during the first few hours after hatching. When this takes place such a ♀ produces fertilised eggs instead of the ♂ eggs which it would otherwise have laid. The layers of fertilised eggs are, therefore, arrenotokous ♀'s, which owe their special nature to the circumstance of having been impregnated by the ♂ during the earlier stages of their growth. And in this connection it is interesting to recall Lenssen's observation that the ♂ egg extrudes a single polar body, whilst the ♀ egg extrudes none.*

Though the thelytokous ♀'s cannot be fertilised they are able to give rise parthenogenetically to arrenotokous ♀'s as well as to other thelytokous ♀'s. The proportion of arrenotokous ♀'s so produced is subject to considerable variation, and to connect these variations with external conditions has been the object of those who have bred these animals with a view to throwing light upon the vexed problem of sex-determination.

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* 'La Cellule,' vol. 14, 1898.

In a series of brief papers communicated to the French Institut, Maupas* held that temperature was the factor which determined the result, but that it produced an effect only in newly-hatched ♀'s before the eggs had commenced to develop in the ovary. To quote his words: "Au début de l'ovogenèse, au contraire, l'œuf est encore neutre et, en agissant convenablement, on peut à ce moment lui faire prendre à volonté l'un ou l'autre caractère sexuel. L'agent modificateur est la température. L'abaisse-t-on les jeunes œufs qui vont se former revêtent l'état de pondeuse d'œufs femelles; l'élève-t-on, au contraire, c'est l'état de pondeuse d'œufs males qui se développe."

More recently Nussbaum† has criticised Maupas' results. He has pointed out that under adverse nutritive conditions the eggs laid by a thelytokous ♀ are often no larger than those laid by an arrenotokous ♀ under normal conditions. Such adverse conditions Maupas must have brought about by raising the temperature, and Nussbaum suggests that Maupas mistook the small ♀ eggs then produced for ♂ eggs. Had he waited for them to hatch he would have realised their true nature. From my own experience, in so far as it goes, I am inclined to attribute some weight to this criticism of Nussbaum, and to agree with him in considering that an element of uncertainty is in this way introduced into Maupas' results.

Nussbaum supported his criticism by experiments and showed that a ♀ subjected to a high temperature before hatching or during the earlier period of its life may give rise to thelytokous ♀'s only (Experiments 21, 23, and 24, pp. 265 to 267).

Having rejected temperature as a factor in the determination of sex in *Hydatina*, Nussbaum turned his attention to other possible influences, and believed that he had found such a one in nutrition.

"Bei *Hydatina senta* bestimmt während einer gewissen Entwicklungsphase die Ernährung das Geschlecht des ganzen Geleges eines jeden jungfraulichen Weibchen. Wird das auskriechende Weibchen bis zur Reifung seines ersten Eies gut ernährt, so legt es nur weibliche Eier; wird es bis zur Geschlechtsreife mangelhaft ernährt, so legt es nur männliche Eier. Vor und nach dieser Periode hat die Ernährung keinen Einfluss" (p. 306).

In such a conclusion I cannot agree with Nussbaum, for reasons which will appear below. It is sufficient to note here that his own experiments do not always bear him out. Thus in Experiment 50, p. 283, he had a "Hungercolonie" on October 4, in which were many ♀ eggs. From the account it is evident that many of these must have hatched and have been without food during the supposed critical early stages before fresh *Euglena* was given them 25 hours later. Nevertheless, no ♂'s appeared in subsequent breeding from the culture.

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* 'Comptes Rendus,' 1890-91.

† 'Archiv f. mik. Anat.,' 1897.

From all this it must be apparent that neither modifications of temperature nor of nutrition afford a satisfactory explanation of the varying proportion of arrenotokous ♀'s which may occur in different cultures of *Hydatina*. There is, however, another explanation of this phenomenon which avoids the necessity of having recourse to external influences. It is possible that it may be the property of certain ♀'s to produce arrenotokous ♀'s in a definite ratio, and again the property of others to produce none. To test such a possibility it is obvious that the starting point for each generation must be the individual and not, as in all of Maupas' and many of Nussbaum's experiments, a collection of individuals.

Methods.—Breeding experiments were started in October, 1904, and, with short breaks, have been carried on until the present time. Each rotifer from which offspring were to be reared was isolated in that form of vessel known as a solid watch-glass, where it lived in about 2 c.c. of water containing *Euglena*. As the young hatched from its eggs they were similarly isolated by means of a fine pipette. If it was desired to test only their immediate offspring, some three or four of these were often placed together for the better economy of space and food. No ♀ was recorded as either thelytokous or arrenotokous until her eggs had been proved to hatch into either ♀'s or ♂'s. The average temperature of the room, as measured by a maximum and minimum thermometer, was about 16° to 18° C. On occasion it was lowered to 6° C. or raised to 24° C. *Hydatina* is extremely hardy and the chief difficulty in breeding it lies in the food supply, for it thrives far better on *Euglena* than on anything else. My supply of this Protozoon was derived from Mr. Thos. Bolton, of Birmingham, and was on the whole regular. In the occasional lean intervals particular experiments came to an enforced end, and my chief care was then to preserve certain strains from extinction. In one instance only was I compelled to have recourse for about 10 days to another food supply, and to make use of the bacteria and other small organisms which occur in a decoction of cabbage leaves. *Hydatina* can live on this diet, but does not thrive as it does upon *Euglena*. A further difficulty in connection with the food supply lies in its purification. The water in which *Euglena* lives usually contains numbers of *Hydatina* with their eggs. To remove these by a pipette is a tedious business, and there is always an element of uncertainty present in that a young rotifer or an egg may have been overlooked. Without doubt some of my earlier experiments are not absolutely trustworthy for this reason, and

I have consequently not made use of them in the following account. No such doubt, however, attaches to the later experiments, for the advent of a small centrifugal machine into the laboratory provided an easy and certain means of obtaining a culture of *Euglena* free of rotifers and their eggs.

Experimental Results.—My experiments have led me to the conclusion that among the rotifers I used were certainly three different types of thelytokous ♀'s, viz.:—

A. ♀'s producing a high percentage of arrenotokous ♀'s.
B. ♀'s producing a low percentage of arrenotokous ♀'s.
C. Purely thelytokous ♀'s producing no arrenotokous ♀'s.

It will be convenient to consider the evidence for this conclusion under these three headings. Incidentally it may be mentioned that the production of ♂'s is, under laboratory conditions at any rate, quite independent of the season of the year.

Type A. ♀*'s Producing a High Percentage of Arrenotokous* ♀'s.

Only once was such a strain met with and bred over a series of generations. The result is shown in Fam. 2 (see diagram, Plate 11). It will be noticed that from the seventh generation onwards the proportion of arrenotokous ♀'s is high and bears to the thelytokous ♀'s a ratio of about 7 : 9, the actual numbers being 46 : 63. Here, as in other experiments, I was unable to trace any order in the sequence of the two types of ♀'s. The proportion of arrenotokous ♀'s among the earlier eggs of a family was neither higher nor lower than among the later ones.

Owing to an unfortunate failure in the food supply the strain was lost before experiments had been made to determine whether it was capable of giving rise to either type B or type C.

Type B. ♀'s *Producing a Low Percentage of Arrenotokous* ♀'s.

It has been my experience that if a ♀ is taken at random and bred from, it will usually turn out to belong to this class. As reference to Fam. 1 (see plate) shows, the percentage of arrenotokous ♀'s is low, but it is not possible to determine it with any degree of accuracy unless very much larger numbers are available. Even such figures may indicate but little, for they may represent merely the resultant of more than one definite ratio.

An interesting feature in this family is the production of an apparently pure thelytokous strain. Commencing with T* we have a series of 15 generations, comprising 177 individuals, in which no arrenotokous ♀ appeared. Failure of food then terminated the experiment, but I think there can be little doubt of a purely thelytokous strain having arisen here. Though arrenotokous ♀'s are rare they yet average 2 to 5 per cent. in families of Type B, and the fact of their non-appearance among 177 individuals seems to be fairly conclusive evidence of the inability of the strain under consideration to produce them.

Type C. Pure Thelytokous ♀'s.

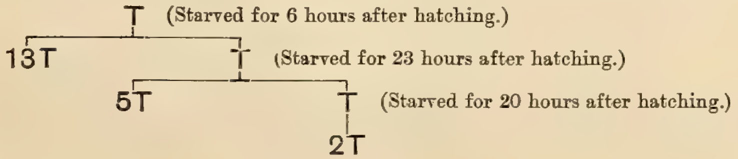
Rarer than Type B, but by no means difficult to obtain, are ♀'s which are incapable of giving rise to arrenotokous offspring. An example of such a strain is shown in Fam. 3, in which 55 generations including 178 individuals were bred and tested without the appearance of a single arrenotokous ♀. In another case 262 thelytokous individuals spread over 24 generations were produced, whilst in a third instance (Fam. 4 of plate) the strain was bred for 73 generations, during which 304 individuals were tested and shown to be purely thelytokous.

In view of these experiments I have referred to the ♀'s of Type C as purely thelytokous. By doing so I have no desire to ignore the possibility that such strains may eventually "break" and give rise to some arrenotokous ♀'s. Since, however, this has not occurred in my experiments the evidence for regarding them as I have done is very strong. In any case the nature of the ♀'s of Type C must be distinct from those of Types A and B.

Influence of Temperature and Nutrition.

It might be argued by some that the continuance of purely thelytokous strains was due to uniform conditions of temperature or of nutrition. Such, however, is not the case. In one of the experiments a purely thelytokous strain was subjected to considerable fluctuations of temperature. During a period of eight days it varied between 8° C. and 11° C., averaging 9° C. The rate of reproduction was much retarded, but in the many subsequent generations produced when the temperature was raised no arrenotokous ♀'s made their appearance. The same strain was afterwards subjected for four days to an average temperature of 22° C. (the extremes being 21° C. and 23° C.) without alteration of its purely thelytokous nature.

A number of starvation experiments were also made, of which the following may serve as an example. An egg from a purely thelytokous strain


T (Starved for 6 hours after hatching.)
13T
T (Starved for 23 hours after hatching.)
5T
T (Starved for 20 hours after hatching.)
2T

(Fam. 3) was isolated soon after being laid and placed in clean water without a trace of *Euglena*. After hatching it was kept without food for six hours and then well fed. It laid 14 eggs which all became thelytokous ♀'s. One of these was again starved for 23 hours and, on being supplied with food, laid six eggs which all gave rise to thelytokous ♀'s. Lastly, one of these six was starved for 20 hours. Though well supplied with food at the end of that time it experienced some difficulty in growing up and laid only two eggs before it died. From both these eggs hatched out thelytokous ♀'s. The relation of these generations is shown in the accompanying scheme. In none of the other similar experiments made with purely thelytokous strains did any arrenotokous ♀'s appear, neither was the proportion of ♂'s raised as the result of starving families of Type B.

Reference was made above to an instance in which, owing to failure in the *Euglena* supply, a decoction of cabbage was used as food. This happened during the 54th and 55th generations of a strain of Type C (see Fam. 4). The animals were poorly nourished and laid very few eggs. Nevertheless the strain just managed to tide over the period of dearth, and on *Euglena* being again forthcoming it went on for 17 further generations before the experiment was stopped. During this time 134 individuals produced were proved to be thelytokous and none were found to be arrenotokous.

In the face of such facts as these it is difficult to entertain the opinion that either temperature or nutrition have any influence in determining the production of arrenotokous ♀'s.

The varying proportion of arrenotokous ♀'s in different cultures of *Hydatina* apparently depends upon the existence of ♀'s of different zygotic constitution, and the solution of the sex problem seems to lie in the determination of the unit-characters involved. The data for such a solution are at present inadequate. Before it is possible to frame a theory we must have further knowledge on the following points:—

(*a*) The number of different types of ♀ that exist as judged by the criterion of their relative output of thelytokous and arrenotokous ♀'s, and the relation of these types to one another.

(*b*) The possible existence of zygotically distinct types of ♂'s and the types of ♀'s from which they respectively arise.

With regard to the former point we have evidence for the existence of (1) purely thelytokous ♀'s, (2) of ♀'s producing arrenotokous ♀'s in the proportion of about 7 in 16, (3) of strains of ♀'s which produce but few arrenotokous ♀'s and in which the rather irregular numbers may be due to the co-existence of two types at present indistinguishable owing to imperfection of analysis, and (4) of ♀'s which, according to Maupas,* produce three arrenotokous ♀'s out of every four. Beyond the fact that (1) may arise from (3) we know nothing of the inter-relations of these various types.

Whether or not there are zygotically distinct types of ♂'s we are at present quite ignorant. The possibility might, however, be tested by a system of fertilisation experiments and subsequent breeding from the ♀'s hatched.

The Nature of Parthenogenesis.—Any interpretation of the facts which is based upon inherent differences in the zygotic constitution of individual ♀'s must necessarily lead us to modify our views as to the nature of parthenogenesis. A given ♀ can be constructed on but a single zygotic plan. Consequently if she produces eggs without the formation of polar bodies, as Lennsen† has suggested, these eggs, provided they contain the somatic number of chromosomes, ought all to possess the same zygotic constitution and the same zygotic properties. But if we assume, and the assumption seems warranted by facts, that external influences are without effect, we must then infer that a ♀ which produces both arrenotokous and thelytokous ♀'s is producing two entirely different kinds of egg. In the light of recent Mendelian research such a condition implies a process of segregation which is probably bound up with a reduction in the somatic number of chromosomes, and the subsequent restoration of that number by the union of two gametic cells each containing the reduced number. Though without evidence either way we have no reason to suppose that the somatic number of chromosomes varies in *Hydatina* during successive generations. We are, therefore, led to suppose that, somewhere antecedent to the formation of an ovum, there is a gametogenic process, and that the recombinations which take place among the gametes so formed determine the proportion of arrenotokous ♀'s. In other words, it seems not inconceivable that the thelytokous ♀'s are really hermaphrodite, though the ♂ gametes may not exhibit the orthodox form of spermatozoa.‡ Such a view would account for the observed absence of polar bodies in the ♀ eggs, for it must be supposed that the process of reduction and fertilisation takes place before the accumulation of yolky material. The ♀ eggs would on this view be comparable, not to the parthenogenetic ♂ eggs, but to ♂ eggs which had undergone fertilisation with the formation of "winter" eggs.

On the other hand it must not be forgotten that, although unable to observe the extrusion of a polar body, Lennsen* nevertheless describes the beginnings of karyokinesis in the parthenogenetic ♀ eggs. The process, however, appears to be arrested half way, and no segregation of chromatic material seems to occur. Whether there is such segregation must be left for future investigation to decide. The problem can be decided only by tracing accurately the fate of the chromosomes, and it is with a view to stimulating further work on the part of the histologist that I have put forward the highly speculative suggestion outlined above.

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* 'Comptes Rendus,' October 6, 1890.

† 'La Cellule,' vol. 14, 1898.

‡ In this connection may be mentioned the interesting discovery of Brauer, that in the parthenogenetic egg of *Artemia* a second polar body is extruded, but immediately re-enters the ovum and fuses with the ♀ pronucleus.

Before concluding these remarks on the nature of parthenogenesis a few words may be devoted to another aspect of the problem. Since the publication of Maupas' experiments on ciliated infusorians it has been generally held among biologists that the effect of conjugation is to arrest a process of senile decay otherwise inevitable in animal stocks which exhibit the phenomenon of asexual reproduction. In other words, there must be a limit to the amount of protoplasm produced in the period between two successive acts of conjugation. Among lowly organisms such as the protozoa this amount may be very large. Among the more highly differentiated metazoa it is relatively small, and where reproduction is entirely sexual it is limited to the amount produced during the lifetime of an individual. A pure thelytokous strain of *Hydatina senta* offers a remarkable contrast to the rest of the metazoa. If we reckon the volume of an individual at 0·01 cubic millimetre, and suppose that each is capable of producing 30 eggs it is a matter of simple calculation to show that the volume of protoplasm producible in 65 generations (*i.e.*, in less than a year) would form a solid sphere of which the dimensions would be such that it could not be contained within the limits of the known universe.† We are, therefore, driven to one of two alternatives—either that what may be termed the potential productivity of protoplasm is immeasurably greater in *Hydatina* than in all other metazoa, or that some process of conjugation occurs at one stage or another in the so-called parthenogenetic reproduction of this form. For reasons already stated I am inclined to favour the latter view, and it is in the hope that the matter may attract the attention of the histologist that I have ventured upon the calculation which forms the substance of this last paragraph.

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* *Loc. cit.*, p. 437.

† For assistance in making the calculations upon which this statement is based I am greatly indebted to my astronomical friend, Mr. Cookson, of Trinity College, Cambridge.

EXPLANATION OF DIAGRAM, PLATE 11.

On the plate are given details of four families of parthenogenetic ♀'s. Fam. 1 contains a few arrenotokous ♀'s, Fam. 2 contains many, whilst Fam. 3 and Fam. 4 show none (*cf.* Types B, A, and C, pp. 226, 227). T denotes a ♀ proved to be thelytokous. A denotes a ♀ proved to be arrenotokous. The small figures in Families 3 and 4 signify the number of the generation from the original parent of the strain. Thus in Family 3, $T_9 + 12T$ means that in the ninth generation 13 ♀'s from the same parent (T_8) were proved to be thelytokous, and that one of these was used as the mother of the tenth generation—the rest being discarded. Where apparent gaps occur, *e.g.*, in Fam. 3, between T_{17} and T_{40}, it means that a single ♀ was used in each generation to continue the strain, but that the sisters of these individuals were not bred from.

On the Julianiaceæ, a New Natural Order of Plants.

By W. Botting Hemsley, F.R.S., F.L.S., Keeper of the Herbarium and Library, Royal Botanic Gardens, Kew.

(Received June 6,—Read June 28, 1906.)

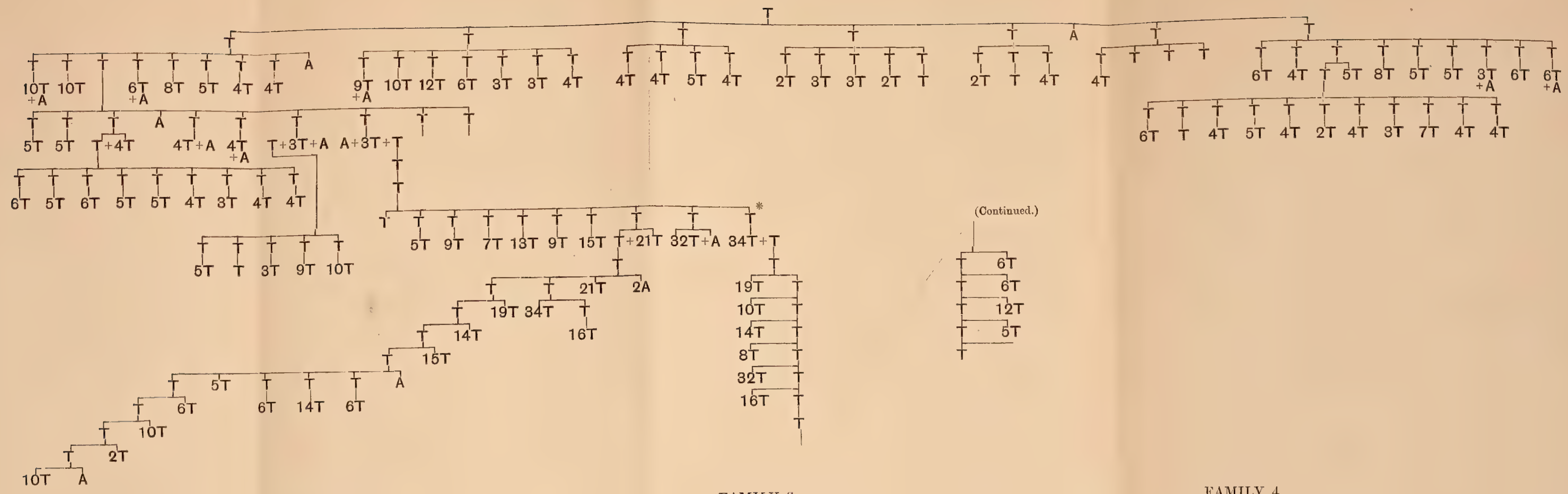
(Abstract.)

I.—General Description.

The Julianiaceæ comprise, so far as at present known, two genera and five species. They are resiniferous, tortuously branched, deciduous, diœcious shrubs or small trees, having alternate, exstipulate, imparipinnate leaves, from about one to three decimetres long, clustered at the tips of the flowering branches and scattered along the short barren shoots. The flowers are small, green or yellow-green, quite inconspicuous, and the males are very different from the females. The male inflorescence is a more or less densely branched axillary panicle or compound catkin, from 2½ to 15 cm. long, with weak, thread-like, hairy branches and pedicels. The male flowers are numerous, 3 to 5 mm. in diameter and consist of a simple, very thin perianth, divided nearly to the base into four to nine narrow equal segments, and an equal number of stamens alternating with the segments. In structure and appearance they are almost exactly like those of the common oak. The female inflorescence is similar in structure to that of the sweet chestnut, consisting of an almost closed, usually five-toothed involucre, borne on a flattened pedicel and containing three or four collateral flowers, of which the two outside ones are, perhaps, always abortive.

At the flowering stage, the female inflorescences, including the narrow

FAMILY 1.


10T +A
10T
6T +A
8T
5T
4T
4T
A
9T +A
10T
12T
6T
3T
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4T
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5T
4T
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3T
3T
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T
A
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4T
5T
8T
5T
5T
3T +A
6T
6T +A
6T
T
4T
5T
4T
2T
4T
3T
7T
4T
4T
5T
5T
T+4T
A
4T+A
4T +A
T+3T+A
A+3T+T
6T
5T
6T
5T
5T
4T
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T
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9T
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9T
7T
13T
9T
15T
T+21T
32T+A
34T+T
*
21T
2A
19T
34T
16T
14T
15T
5T
A
6T
6T
14T
6T
10T
2T
10T
A
19T
10T
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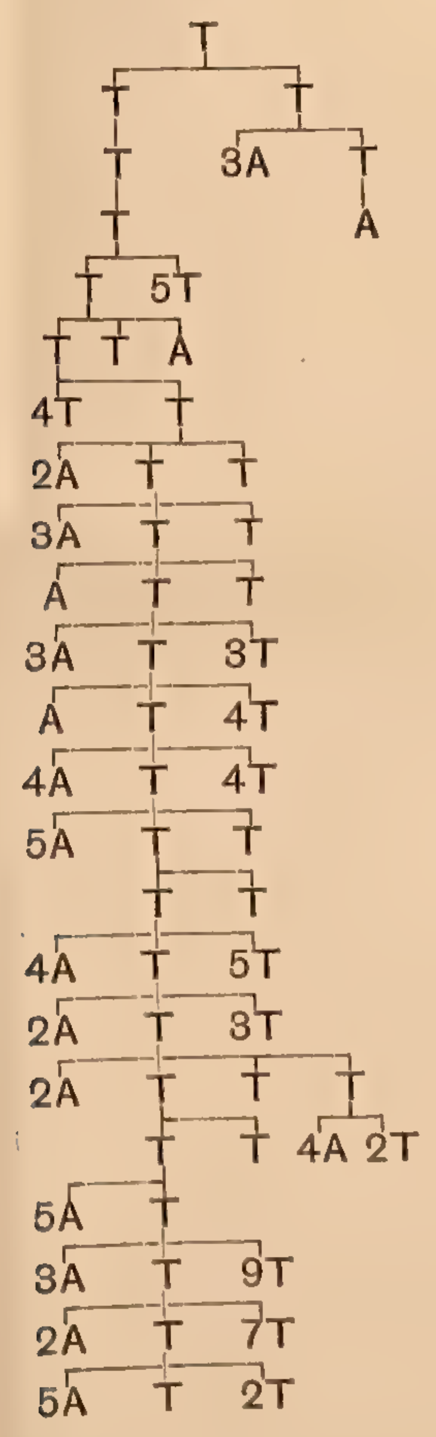
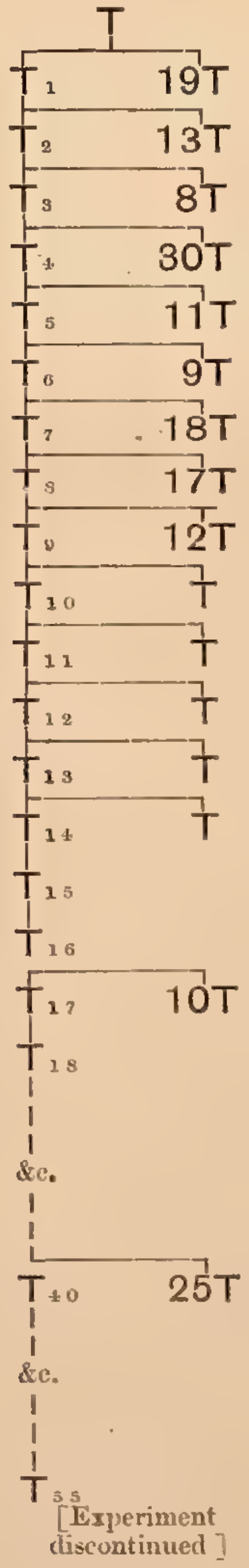
8T
32T
16T
(Continued.)
6T
6T
12T
5T

FAMILY 2.


3A
A
5T
A
4T
2A
3A
A
3A
3T
A
4T
4A
4T
5A
4A
5T
2A
3T
2A
4A
2T
5A
3A
9T
2A
7T
5A
2T

FAMILY 3.


1
19T
2
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11T
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7
18T
8
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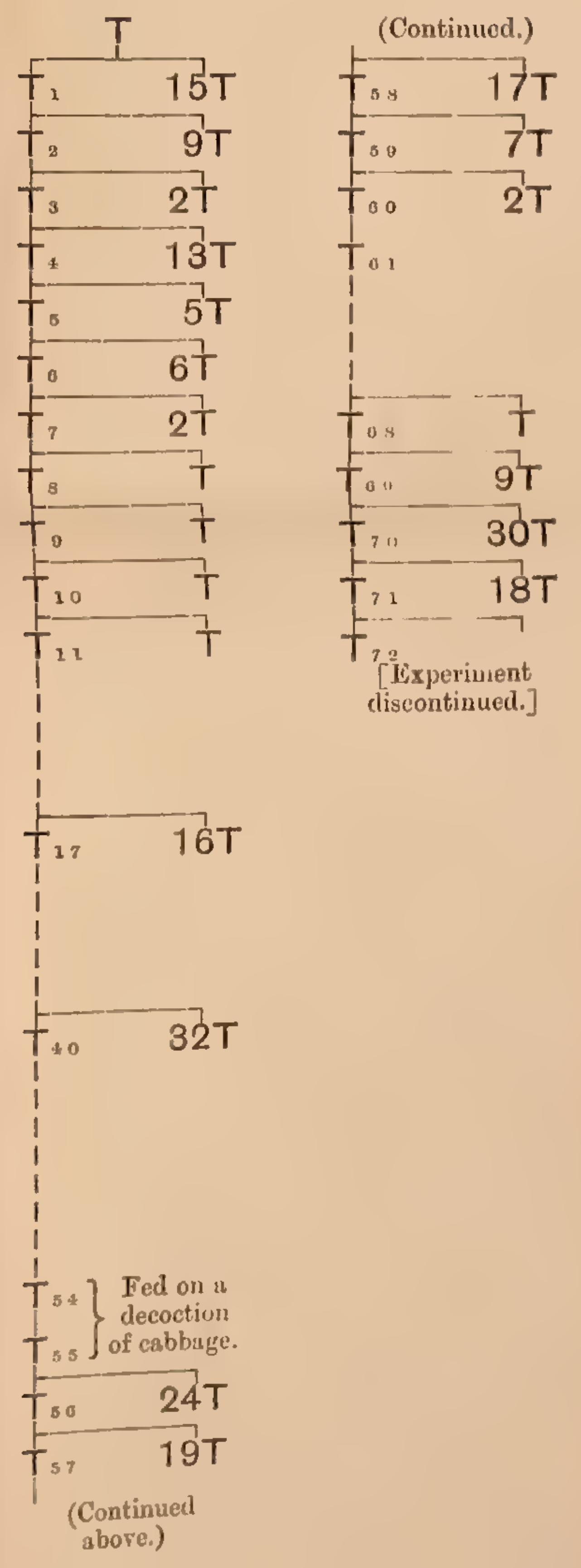
9
12T
10
11
12
13
14
15
16
17
10T
18
&c.
40
25T
&c.
55
[Experiment discontinued.]

FAMILY 4.


1
15T
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13T
5
5T
6
6T
7
2T
8
9
10
11
17
16T
40
32T
54
55
Fed on a decoction of cabbage.
56
24T
57
19T
(Continued above.)
(Continued.)
58
17T
59
7T
60
2T
61
68
69
9T
70
30T
71
18T
72
[Experiment discontinued.]

flattened pedicel and the exserted styles, are about 2 cm. long, and as they are seated close in the axils of the crowded leaves, and of the same colour, they are easily overlooked. The female flowers are destitute of a perianth, and consist of a flattened, one-celled ovary, terminated by a trifid style and containing a solitary ovule. The ovule in both genera is a very peculiar structure. I will first describe that of *Juliania.* In the flowering stage it is a thin, flat, obliquely horseshoe-shaped or unequally two-lobed body, about 2 mm. in its greatest diameter, attached to the base of the cell. At a little later stage, in consequence of unequal growth, it is horizontally oblong, nearly as large as the mature seed, that is 6 to 8 mm. long, and almost symmetrically two-lobed at the top. A vascular bundle or strand runs from the point of attachment to the placenta upwards near the margin into one of the lobes. In this lobe the embryo is tardily developed, and at this stage it is more or less enclosed in the opposite lobe, the relations of the two being as nozzle and socket to each other. It is assumed that the whole of this body, with the exception of the lobe in which the embryo is formed, is a funicle with a unilaterally developed appendage, which breaks up and is absorbed during the development of the ovule into seed. A similar growth and transformation is unknown to me in any other natural order.

The ovule of *Orthopterygium* is very imperfectly known, but the attachment appears to be lateral and the funicular appendage cup-shaped at the basal end, bilamellate upwards, and more or less enclosing the embryoniferous lobe.

Mr. Boodle, who has fully examined the ovule of *Juliania* from microtome sections, describes it as hemianatropous with a single integument.

The compound fruits of *Juliania* are samaroid in form, the wing being the flattened pedicel, at the base of which it disarticulates from the undifferentiated part of the pedicel. They vary from 4 to 7 cm. in length by 1½ to 2½ cm. in width. Externally they strongly resemble the samaroid pods of certain genera of Leguminosæ, notably those of *Platypodium* and *Myroxylon.* The involucre itself, of the largest fruits seen, is only about 1 cm. deep by 2 cm. wide. It is composed of very hard tissues and is quite indehiscent. Only quite young fruit of *Orthopterygium* is known. In this the flattened pedicel is narrow, straight and æquilateral, from 6 to 7 cm. long and about 1 cm. wide.

The nuts of *Juliania* are almost orbicular, biconvex, hairy on the outside and have a very hard endocarp. The solitary exalbuminous seed is circular or oblong, 6 to 10 mm. long, compressed, with a smooth, thin testa. The embryo is horizontal, with thin plano-convex, more or less oblique, obscurely lobed cotyledons, which are epigæous in germination, and a long ascending radicle applied to the edges of the cotyledons.

II.—History.

It is surprising that a genus of plants so striking in aspect, so distinct in the shape of its fruit, and so widely spread as *Juliania* is in Mexico, should have entirely escaped the observation of all the earlier European travellers in that country.

C. J. W. Schiede, M.D., who accompanied Ferdinand Deppe on a botanical expedition to Mexico in 1828, was apparently the first to send dried specimens to Europe of one of the species of *Juliania*. But it was not until 1843 that his friend, Dr. D. F. L. von Schlechtendal, published an account of the genus of plants in question.

Under the name of *Hypopterygium* (subsequently *Juliania*) *adstringens*, he very fully described the material he had an opportunity of examining, but he had neither female flowers nor mature seeds, and he was doubtful whether the fruit was the result of one or more flowers. His description is very accurate, and he expresses his views of the affinities of the plant, which he regarded as the type of a new Natural Order. Since Schlechtendal's time, until I took up the study of the genus five years ago, nobody seems to have had sufficient material to supplement his description.

In 1854, A. Gray described, also from very incomplete material, what he considered a second species of the same genus, collected in Peru. An examination of fuller, though by no means complete, material has led me to separate it generically under the name of *Orthopterygium*.

In September, 1900, the late Mr. Marc Micheli presented Kew with a small set of E. Langlassé's Mexican plants. Among them was a specimen in fruit, which, after much research, was identified with Schlechtendal's *Juliania adstringens;* but the most careful and tedious examination carried me no further than Schlechtendal had reached 60 years before. Previous to this (in 1899, as I afterwards found out), Kew received a specimen of a male plant collected in the Mexican State of Jalisco by Mr. C. G. Pringle, n. 6871, and doubtingly named *Juliania adstringens*.

The male specimen was published as *Juliania mollis*, Hemsl., and the fruiting as *J. adstringens*, Schl.

This publication had the desired effect, for it brought me a letter at the end of 1901 from Dr. J. N. Rose, Curator in the "Division of Plants" of the United States National Museum at Washington, from which I make the following extracts:—

"You will also be interested in what I have to tell you about *Juliania*. For more than six years I have been at work off and on, at this genus, but for the lack of material I have never published anything upon it, but each time have brought back specimens, and this year was especially fortunate in collecting near the type-locality both male and female plants. In looking up the subject since my return I find that you have anticipated me and have published two very beautiful plates and some interesting notes. . . . There are, however, more than two species in Mexico. I have certainly four well-marked species and possibly six. . . . With regard to the position of this genus, I think it must be regarded as the type of a new order. I do not think it has any relationship to either Burseraceæ or Anacardiaceæ. My conclusions in the field were that it must be closely related to Juglandaceæ, a relationship which you also suggest."

In this communication Dr. Rose most generously offered to send all his specimens and notes to me, leaving it to my judgment in what form publication should be effected. I gladly accepted, and through the kindness of the Trustees of the Bentham Fund, Miss M. Smith made an elaborate series of drawings under my direction. As there were still some structural points on which we were not quite clear, and Dr. Rose contemplated another visit to Mexico, it was decided to publish at once a description of the genus, as then understood, and brief diagnoses of the species.

III.—GEOGRAPHICAL DISTRIBUTION.

1. *Juliania.*

So far as at present known, *Juliania* is confined to Mexico, and the various species occur in isolated localities between about 17° 40′ and 23° N. lat., and 97° and 104° W. long., and at altitudes of about 1500 to 5500 feet.

2. *Orthopterygium.*

The habitat of the Peruvian *Orthopterygium Huaucui* is 2000 miles distant from the nearest locality of any species of *Juliania*. The exact position of the only place in which it has been found cannot be given, but it is in the Province of Canta, in the Department of Lima, between 11° and 12° S. lat.

IV.—THE AFFINITIES OF THE JULIANIACEÆ.

During the six years that I have had this small group under observation I have had opportunities of showing the specimens and drawings to many of the leading botanists of the world, and all agree who have seen them that it deserves to rank as an independent order. That being so, the question of its position arises, but that is a point not so easily settled in a linear arrangement. Taking the morphological characters seriatim, it is evident that the closest relationships are with the Anacardiaceæ and Cupuliferæ. The absolute separation of the sexes and the very great diversity of the floral structure of the sexes, associated with pinnate leaves, offers a combination of characters probably without a parallel.

Beginning with the foliage, the Julianiaceæ have alternate, exstipulate, imparipinnate leaves in common with at least eight different ligneous orders, but here the affinity, or, rather, resemblance ends so far as six of them are concerned, and the comparisons need be carried no further. There remain the Anacardiaceæ and Juglandaceæ, both of which are also resiniferous, both have unisexual flowers with reduced envelopes, at least as to some of their members, and both have solitary, exalbuminous seeds. Other points of resemblance or similarity in the Juglandaceæ are the dissimilar male and female flowers, the broad, stigmatic lobes of the style, and the single-coated ovules. *Juglans* has also a funicle of unusual development. But the combined characters in common of the Julianiaceæ and the Juglandaceæ cannot be regarded as constituting a close affinity. In some respects there is a nearer relationship to the Anacardiaceæ. The anatomical characters of the two orders are very much alike; but as Dr. F. E. Fritsch will describe and discuss the anatomy in a separate paper, it is unnecessary to enter into particulars here.

The nearest approach I have found to the singular funicular development of the ovule is in the Anacardiaceæ, but the resemblance is remote and the ovules of the latter are double coated. Coming to the seed and the embryo, however, the resemblance is complete, and, apart from the slight obliquity of the cotyledons of *Juliania*, the description of the seed and embryo of *Cotinus* or *Rhus* would do for *Juliania*. With this the affinities to the Anacardiaceæ are exhausted, and they are not sufficiently strong to justify the juxtaposition of the two orders. The next comparison is with the Cupuliferæ, taking the order as limited by Bentham and Hooker. There is nothing in the secretions nor in the foliage to warrant an approximation of the two orders, and in habit of growth the Julianiaceæ are very different. But divergences as great, or greater, exist between closely associated orders, and even between genera referred to the same order; and when we come to the inflorescence and flowers, affinities are evident; that is if affinities are deducible from similarities in structure.

The male inflorescence, the male flowers, and the pollen of *Juliania adstringens* are so near in texture, structure, and form to the same parts in certain species of oak that, detached, they might be referred to the genus *Quercus*. In fact, there is much greater dissimilarity in the male inflorescence and flowers of different species of *Quercus* than there is between those of *Juliania* and those species of *Quercus* which have a flaccid male inflorescence and stamens alternating with the segments of the perianth.

The female inflorescence and the male flowers of *Juliania* are not represented by exact counterparts in the Cupuliferæ, but the analogies are perhaps greater than with any other order. Several female flowers in a closed involucre is a characteristic of *Juliania*, of *Fagus*, *Castanea*, and *Castanopsis*. In all three of the genera of the Cupuliferæ named, the involucre dehisces regularly or irregularly, and the nuts fall out. In *Juliania* the involucre is indehiscent, and the flattened nuts are adnate by their edges to the inner wall of the involucre, and they have a very hard, relatively thick, sclerenchymatous pericarp.

Going back to the flowers, the male of *Juliania* has a perianth; the female none. In *Corylus* the conditions are reversed; in *Betula*, neither sex has an obvious perianth; in *Quercus*, the flowers of both sexes are furnished with a perianth.

All of the Cupuliferæ have an ovary which is more than one-celled, and usually there are three cells, and mostly more than one ovule in each cell, though each nut is usually only one-seeded. The ovary of *Juliania* and of *Orthopterygium* invariably contains only one ovule. The flowers and nuts of *Castanea* are collateral, as in *Juliania*. The seeds of both orders are exalbuminous, and the cotyledons are epigæous in germination.

Weighing the characters in which there is agreement or similarity between the Julianiaceæ and the Anacardiaceæ, and those in which there is agreement or similarity between the Julianiaceæ and the Cupuliferæ, the latter in my estimation preponderate; and I cannot suggest a more natural position for the Julianiaceæ, in a linear arrangement, than between the Juglandaceæ and the Cupuliferæ.

Communication on Regeneration of Bone.

By Sir WILLIAM MACEWEN, F.R.S.

(Received June 6,—Read June 7, 1906.)

The following experiments, illustrative of the part played by the various structures in the regeneration of bone, were performed upon young animals of two different varieties of the canine species, which were still in their developmental period, two to four months. The specimens were secured from 6 to 12 weeks after the experiments had been performed, consequently the animals would then be from three to six months old.

A.—The periosteum removed from the entire circumference of the right radius, the bone remaining *in situ.*

B.—A circle of periosteum removed from the entire circumference of the shaft of right radius and a silver ring placed upon the denuded bone.

D and E.—Bone grafting from right radius.

F and G.—Transplantation of bone *en masse* from right radius.

J.—Preservation of periosteum. Removal of part of shaft of right radius. Protection of divided ends of bone and medullary tissue by decalcified turkey bone.

K.—Preservation of periosteum and removal of underlying bone of right radius to test the osseous reproductive capacity of this membrane.

A.—*The Periosteum Removed from the Entire Circumference of the Right Radius, the Bone Remaining* in situ.

Dog A had the periosteum removed from the entire shaft of the right radius, leaving only $\frac{1}{4}$ inch of periosteum on the diaphyseal side of the epiphyseal lines.

The operation was practically bloodless, aseptic healing ensued without visible scar or adherent cicatrix. Along with this specimen there is the corresponding left radius for comparison.

Description of specimen as seen about 12 weeks afterwards:—

The shaft of the bone was found to be entirely covered with a layer of newly formed connective tissue, closely investing the bone and adhering to it much more firmly than periosteal tissue. It was found more difficult to detach this fibrous layer from the bone than normal periosteum. The bone was quite healthy and had acquired an abundant new blood supply. It had, however, not increased circumferentially to the same extent as its fellow on the left side.

B.—*A Circle of Periosteum Removed from the Entire Circumference of the Shaft of Right Radius and a Silver Ring placed upon the Denuded Bone.*

Dog B had a circle of periosteum, comprising the whole circumference of the right radius and measuring $\frac{1}{2}$ inch in breadth, raised from about the middle of the shaft. A flattened silver ring was made to encircle the denuded bone at this part.

The operation was bloodless, the wound healed aseptically without visible scar and without adherent cicatrix. The bones of the left limb are preserved for comparison.

Description of specimen as seen 12 weeks after :—

The right radius was covered—at the part that had been denuded of the periosteum—with a newly formed connective tissue which was more firmly attached to the bone than that of the normal periosteum on the shaft above and below this part. After denuding the bone from this newly formed connective tissue and from the periosteal covering, there was no trace of the silver ring to be seen. The shaft of the bone was smooth all over and if the wire still existed it must have become enveloped in the bone. There was, however, a thickening of the shaft at one part and it was considered probable that the silver ring lay underneath. The bone was scraped through in a vertical direction and after penetrating the bone for about $\frac{1}{8}$ inch, the silver ring was exposed, completely buried in firm osseous tissue. Three other apertures were scraped through at different parts of the circumference of the shaft so as to expose the silver ring at each. The thickness of the new bone covering the wire in front was fully $\frac{1}{8}$ inch and it was a little less behind. Thus, the silver ring placed upon the bone denuded of periosteum had in three months become completely enveloped in newly formed bone, $\frac{1}{8}$ inch in thickness.

The shaft operated on is smaller in circumference than its fellow in left limb, the diminution in bulk being most apparent at the part which was denuded of periosteum—but the new bone at this part is denser than that on the other parts of the shaft which were not operated on.

D and E.—*Bone Grafting.*

Two dogs, named D and E, of different species, had the periosteum entirely removed from the right radius, after which a circle of bone including the whole circumference of the shaft was removed from each. These circles of bone being kept apart were each divided into small fragments, and those removed from animal D were placed in the gap left in the radius of E, while those from animal E were placed in the gap left in the radius of D.

No bleeding, aseptic healing without visible scar. Osseous union solid at end of fifth week. The left fore limb of each is preserved for comparison.

Description of specimens as seen 12 weeks after transplantation of bone grafts:—

Right Radius of D.—Layer of fibrous tissue covering whole of shaft of right radius and so firmly attached that scalpel as well as periosteal elevator were required to denude the bone. Shaft is entire and continuous from one epiphysis to the other, but there is great thickening at the part where the graft from neighbouring bone was introduced. In marked contrast to the dense, firmly adherent new formation of connective tissue surrounding the right radius on this limb was the thin periosteal covering of the left radius which shelled with great ease on the application of the periosteal elevator.

Description of specimen E is very similar to that of D.

Right radius of E.—Dense layer of new formation of fibrous tissue covering bone and firmly adherent to the shaft. Shaft united throughout, but presents large irregular node at seat of bone grafting.

F and G.—*Transplantation of Bone* en masse.

Two dogs, F and G, of different species had the periosteum entirely removed from the right radius, after which the greater portion of the shaft of the right radius, extending from near the proximal to near the distal epiphyses was removed *en masse* and transplanted, the shaft of the right radius of F being inserted into the gap in the shaft of G, and the shaft of the right radius of G being introduced into the gap in the radius of F.

No vessels requiring ligation. Aseptic healing, leaving no visible or adherent scar. Bone firm at end of four weeks, and subsequently increase in circumference was in F detected through soft tissues. The left radii are preserved for comparison.

Description of Specimens.—Right radius of F seen 11 weeks and 1 day after transplantation. New formation of dense connective tissue closely adhering to the bone and with difficulty elevated therefrom. The shaft, continuous with the epiphyses and firmly united thereto, is greatly increased in circumference, and is much thicker than the radius of the left limb from which the normal periosteum is easily raised in one piece by the elevator.

G.—Right radius of G seen 20 weeks and 4 days afterwards. Newly formed connective tissue closely adherent to the transplanted shaft. The diaphysis is united throughout, and so perfect is the union at either end that the insertion can scarcely be made out. It looks like a well formed normal shaft.

Dog J.— *Preservation of Periosteum, Removal of Part of Shaft and Protection of Medullary Tissue and Divided Ends of Bone.*

The periosteum from the right radius was elevated, carefully preserved and left *intact.* There was no attempt made to free the periosteum from plaques of bone which may have adhered to it during separation. 2⅝ of the denuded shaft of the right radius was then removed and put aside. In its place a perforated, decalcified turkey bone drainage tube was inserted into the gap in the shaft under the periosteum. The proximal and distal portions of the shaft with their open medulla were introduced into either end of the turkey bone tube with the hope that any extrusion of ossific matter, either from the divided ends of shaft or from the medulla, would be thus preserved from the pressure of the surrounding tissues and be permitted to grow.

Almost bloodless. Aseptic healing, no visible scar and no adherent cicatrix. Dog ran about a great deal, and ulna became bent from lack of support of radius.

Description of Specimen as seen 10 *Weeks after.*—The ulna was found bent at junction of middle and lower thirds. In the part of the radius from which the shaft had been removed there was no apparent trace of periosteum or decalcified turkey bone tube, but where the tube had been placed there was a dense mass of fibrous tissue about ¼ inch in thickness, which covered the underlying osseous tissue, and adhered to the ulnar periosteum. This connective tissue mass was continuous superficially with the periosteum on the shaft above and below the part from which the bone had been removed, while at its deeper part it was connected with the two extremities of the old shaft and with portions of new bone projecting from the cut edges of the old, which had formed an irregular bridge between the two cut osseous surfaces, measuring 1½ inches in length, and from ¼ to ⅜ inch in breadth. On the proximal side of this bridge there was a very thin osseous layer adhering to the ulna, and to the upper part of radius, the remainder of the gap being made up of dense fibrous tissue.

The difference in length between the right radius and the left radius is ¾ of an inch—the measurement being in a straight line (5½ left; 4¾ right).

Dog K.—*Preservation of Periosteum and Removal of Bone.*

Shaft of radius removed sub-periosteally to the extent of 1¾ inches; the periosteum being carefully preserved and being left *in situ.* There was no attempt made to detach plaques of bone which might have adhered to the periosteum during its separation. At the upper extremity of the bone shaft, an irregular spike-like process of bone was left. The wound was then closed, the soft tissues being allowed to coalesce. A case of plaster of Paris was applied loosely to prevent outward pressure.

No bleeding. Aseptic wound healing, leaving no visible cicatrix, and no adherent scar. After removal of plaster four weeks subsequently, there was no apparent union, a gap being detected between the extremities of the radius. At end of six weeks gap quite marked. Ulna markedly bending.

Examination of Specimen 10 *Weeks After.*—The right ulna had markedly bent, and this bending had lessened greatly the interval which would otherwise have existed between the two extremities of the un-united radius. At the part from which the bone had been removed and the periosteum had been left *intact* there was a gap, void of osseous formation, but filled with dense connective tissue. When this was turned aside, the proximal portion of the bone was seen to be flattened laterally, a new formation of bone continuous with the shaft projecting toward the gap A somewhat similar formation had taken place below.

ERRATA.

Proceedings No. B 523.—Paper by Miss M. P. Fitzgerald.

Page 96.—The heading (b) should have been placed in middle of page 95, above par. beginning "The section area . . . "

Page 100.—The heading (d) should have been placed on page 99, above par. beginning "The section area . . . "

Page 102.—The heading (e) should have been placed on page 101, above par. beginning "The section area of . . . "

Page 106.—The italic heading should have been placed in middle of page 105, above italic heading (a).

Page 109.—The heading (a) should have been placed on page 108, above par. beginning "Both the sectional area . . . "

Page 110.—The heading (b) should have been placed on page 109, above par. beginning "The sectional area . . . "

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Glossina palpalis *in its Relation to* Trypanosoma gambiense *and other Trypanosomes* (*Preliminary Report*).

By E. A. Minchin, M.A., Professor of Protozoology in the University of London, Lieutenant A. C. H. Gray, R.A.M.C., and the late Lieutenant F. M. G. Tulloch, R.A.M.C. (Sleeping Sickness Commission).

(Communicated by the Tropical Diseases Committee. Received July 10, 1906.)

[Plates 12—14.]

Introductory.

In this paper we propose to give a brief statement of the results obtained by us with reference to the relation of the tsetse-fly (*Glossina palpalis*) to the trypanosome of Sleeping Sickness (*Trypanosoma gambiense*), and to other species of trypanosomes which this fly carries.

Our investigations have consisted of observations and experiments upon (A) Flies fed in the laboratory on animals which had been infected by the inoculation of cerebro-spinal fluid from sleeping sickness patients, and which showed trypanosomes in their blood as the result of such inoculation. (B) Flies caught in various localities which were found on dissection to contain trypanosomes in their digestive tracts.

Of the trypanosomes under the latter heading (B) we have found two distinct types. One of these types has been named by Professor F. G. Novy* *Trypanosoma grayi*, and one of us (Professor E. A. Minchin) proposes to call the other type *Trypanosoma tullochii*.

Type I. *T. tullochii*, n. sp. Minchin.—This type is distinguished by its more rounded nucleus placed near the middle of its body, by a small, usually circular, blepharoplast placed well behind† the nucleus, *i.e.*, at the end furthest from the flagellum (Plate 14, figs. 53—60).

Type II. *T. grayi*, Novy.—This form is characterised by its large nucleus, which may be oval, spherical, or compressed, and which is in all cases situated not far from the posterior end of the body. In many cases the nucleus shows distinctly eight chromosomes. The blepharoplast is large, transversely elongated, and situated close to the nucleus, either at its side or more usually anterior to it. Sometimes, however, it may be posterior to the nucleus, a point which we discuss further below. The flagellum is often distinctly thickened at the tip. This type varies very greatly both in form and size. We distinguish (1) male forms, very slender, with long free flagellum, with nucleus very compressed, and with the blepharoplast situated in front of it (Plate 13, figs. 21 and 22). Some of the forms reach an extraordinary length (Plate 13, fig. 33). (2) Female forms which are bulky, often thickened at the posterior end and with an oval or rounded nucleus. The blepharoplast is variable in position, and the free flagellum is very short (Plate 13, figs. 23—25 and 34). (3) Young forms (Plate 13, figs. 31 and 32) and indifferent forms, varying greatly in character; among the latter we may particularly note forms which are nearly spherical (Plate 14, figs. 43—51). The very protean character of these forms (see Plate 13, figs. 35—40; Plate 14, figs. 41—52) makes it very uncertain as to whether they are really all of the same species. Since, however, we have noticed a marked difference between trypanosomes from flies which had fed after being caught and those in flies which had not fed (Plate 13, figs. 33 and 34, and Plate 14, fig. 41), we think that these variations of type are to be explained as the result of the conditions of nutrition of the host. The forms from flies which had not been fed were both scarcer and larger than those from flies which had recently sucked blood. In flies dissected soon after feeding it was found that small forms (Plate 13, figs. 31 and 32) largely predominated, and dividing forms were numerous (Plate 13, figs. 27, 28, and 29); on the other hand, in those cases in which flies were found to contain forms of a more indifferent character (Plate 13, figs. 36—40), it was noticed that stages of division were extremely rare, and that aggregations of similar forms into large masses were frequent (Plate 14, fig. 42).

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* 'Journal of Infectious Diseases,' vol. 3, No. 3 (May, 1906), pp. 394—411, Plates 15—17. Professor Novy gives some excellent microphotographs of these trypanosomes, taken from preparations sent him by Lieutenant Gray.

† In this memoir we use the terms anterior and posterior purely with reference to the direction of locomotion of the trypanosomes described by us, and without prejudice to the disputed morphological questions involved.

The mode of division in *T. grayi* is noteworthy and characteristic. The two sister individuals which result from it are markedly unequal in size and differ also in the relations of their nucleus and blepharoplast. The smaller of the two has the blepharoplast placed in what may be considered the normal position, that is to say, well in front of the nucleus. On the other hand, the larger individual has the blepharoplast placed behind the nucleus (Plate 13, fig. 28). We consider, therefore, that the forms not infrequently found, in which the blepharoplast is situated behind the nucleus, represent, in many cases at least, the larger of two sister individuals resulting from recent division. Multiplication by division has only been observed by us in individuals of indifferent or female type, never in fully differentiated male forms. Finally, we may draw attention to the numerous chromidia always present in young, indifferent, or female forms.

In their staining reactions the chromidia seem to resemble the blepharoplast more than the nucleus.

(A) *Observations and Experiments with Flies Artificially Infected with* T. gambiense.—We undertook very numerous experiments to determine the exact mode of infection by the fly, particularly with the object of determining whether the fly became infectious at any definite period after having been fed on an infected animal. For instance, a batch of freshly caught flies was fed first on an infected animal, and then fed on successive days on a series of healthy animals, using a fresh animal for each feed, the experiments covering a period of 22 days from the time of the original infection of the flies. All such experiments, however, gave entirely negative results. On the other hand, we obtained positive proof that *G. palpalis* can convey trypanosomes by means of its proboscis from an infected to a healthy animal, if it goes straight from the one to the other. Our method of experimenting was as follows: A single fly was placed in a test-tube and the mouth of the tube covered with gauze. The mouth of the tube was then pressed on to the infected animal and the fly carefully watched. When the fly had about half fed it was removed from the infected animal and placed on a healthy one, on which it was allowed to finish its meal. Infection by trypanosomes was effected by this means in four out of five experiments when *G. palpalis* was used as the transmitting agent, and once out of four experiments when a *Stomoxys* was used in a similar manner. In order to determine further whether in these cases the infection was brought about by contamination with the fly's proboscis only or by the possible regurgitation of already ingested trypanosomes from the digestive tract, a further series of experiments was carried out, in which the fly, after having partially fed on an infected animal, was then allowed to feed on two healthy animals in succession. Five such experiments were carried out, in each of which it was observed that the fly (*Glossina*) had sucked blood from both the infected and the two healthy animals. In every case the *first* of the two healthy animals, and only the first, was infected even when the fly had only been allowed to dip its proboscis for a moment into the first healthy animal and was then immediately transferred to the second healthy animal. This shows, in our opinion, that the infection is conveyed by contamination of the proboscis, and that if the fly be allowed to clean its proboscis by piercing the skin of one animal, it is no longer infectious to a second. In these experiments upon direct transmission the "Jinja" cattle-trypanosome was used by us, because it is abundant in the blood of infected animals (rats) with which we were working, and also on account of the fact that the infection or non-infection of a rat with this trypanosome is a matter of certainty within a very few days, whereas had we used *T. gambiense* the results of our experiments would have remained uncertain for a very long while.

It has also been proved by the experiments of Bruce and by ourselves that freshly caught specimens of *G. palpalis,* at Entebbe, are capable of infecting animals with the trypanosome of Sleeping Sickness, but in this case all experiments seem to show that the number of fly-bites required to produce infection is a very variable one indeed, since over and over again more than 1000 flies have fed on a susceptible animal without infecting it. The smallest batch with which we ourselves have been successful in producing infection consisted of 134 flies.

Observations on the fate of trypanosomes introduced into the digestive tract of the tsetse-fly by feeding it in the laboratory upon animals infected with *T. gambiense* gave the following results:—The trypanosomes, never very numerous in the ingested blood, show at the end of 24 hours a slight increase in number, and many of the parasites are observed in stages of division. At the same time they have become differentiated into two very distinct forms. The first is a very slender type with cytoplasm free from granules, with the nucleus sometimes rounded but more usually compressed, and with a considerable length of free flagellum (Plate 12, figs. 1 to 6). Many of these slender forms are observed at this stage to be in the act of extruding granules of chromatin from the nucleus (Plate 12, figs. 4 to 6). The second form of parasite is relatively very large and bulky with granular and deeply-staining cytoplasm, with very large spherical nucleus, with short free flagellum, and with the blepharoplast often some distance from the posterior end (Plate 12, figs. 7 to 14). These two forms may be regarded, on the analogy of developmental facts recorded of other trypanosomes, as male and female respectively. In both forms stages of division were observed, but in no case have we succeeded as yet in observing with certainty any process of conjugation. The two forms are easily distinguished in the living condition, the slender males being also characterised by much greater activity than the bulky females.

Male and female forms could also be recognised in the blood of the experimental animals (monkeys), especially in films fixed with osmic vapour. In films dried in the ordinary way the characteristic differences were much less distinct. In either case the differentiation of sexual characters is far less marked than it becomes in the intestine of the fly. Trypanosomes of male character (Plate 12, fig. 16) are common in blood-films, but those of female character (Plate 12, fig. 17) are very scarce, and only two were found, both of which were remarkable for having the nucleus composed of four distinct masses of chromatin. On the other hand, an abundant form in the blood films is an indifferent type (Plate 12, figs. 18 and 19), characterised usually by very short free flagellum, and it is this form which develops into the female form in the fly. In this connection attention should be drawn to the forms, distinctly of the female type, obtained by two of us (Gray and Tulloch) in a culture (Plate 12, fig. 20, see Appendix II).

It may be pointed out that the sexual forms of *T. gambiense* from the tsetse-fly are very similar to the forms of *T. brucii* described by Koch* from other species of *Glossina,* so far as can be judged from Koch's figures. It is our opinion, however, that many of the forms described by Koch as developmental stages of *T. brucii* are really stages of one or more distinct species of trypanosomes carried by the flies, comparable to, and perhaps identical with, *T. grayi* and *T. tullochii* in *G. palpalis.*

At 48 hours after feeding the trypanosomes are still numerous in the intestine of the fly, and a type of more indifferent character begins to make its appearance (Plate 12, fig. 15). At 72 hours the trypanosomes are usually beginning to become more scanty and difficult to find in the digestive tract of the fly, although in some cases they are still numerous and chiefly of the indifferent type. At 96 hours, in almost every case, not a single trypanosome could be found even after the most careful searching. In one case a single trypanosome was found, and in another case two, on the fourth day, but in all other cases the trypanosomes seemed to have vanished completely at this period, and could never be found at any subsequent time. It would appear as if they died out with the absorption of the blood with which they were ingested, and were unable to pass forward in the digestive tract into the blood taken up by the fly at any subsequent feeding. In this they contrast sharply with the trypanosomes described above, occurring in the fly under natural conditions.

The disappearance of *T. gambiense* from the digestive tract of the tsetse-fly could be interpreted in one of three ways: (1) the trypanosomes may actually die out and be digested; (2) the trypanosomes may pass from the digestive tract into other organs of the fly; (3) the trypanosomes may become, by rapid division, so minute as to escape detection, like the forms of *Spirochæta ziemanni* described by Schaudinn, or like the invisible micro-organism of yellow fever. In order to test the second of these two possibilities, the internal organs of a number of artificially infected flies were carefully examined, but always with negative results, while the experimental results of Bruce and ourselves seem to disprove infectivity of the fly at any period after 48 hours, and, therefore, render improbable the third possibility suggested above. So far then as it is possible to draw conclusions from our observations, it would appear that *T. gambiense* does actually die out in the tsetse-fly after the third day. In all cases *T. gambiense* was found only in the mid-gut of the fly, and appeared never to pass either backwards into the proctodæum or forwards into the proventriculus, another point in which they contrast with the "fresh fly trypanosomes."

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* 'Deutsch. Med. Wochenschr.,' 1905, No. 47.

(B) *Observations and Experiments upon Freshly-caught Tsetse-flies Found to Contain Trypanosomes.*—When freshly-caught tsetse-flies were examined by us in the laboratory, either after having been fed upon a healthy animal or not, a certain percentage of them were found to contain trypanosomes of one or rarely of both types referred to above as *T. grayi* and *T. tullochii.* In such cases the trypanosomes were usually present in enormous numbers, especially if the fly had been previously fed. These trypanosomes, when compared with *T. gambiense* artificially introduced into a fly's intestine, are distinguishable by their appearance and movements. They are far more active than the sluggish *gambiense,* especially the male forms, which often shoot across the field of the microscope with the greatest rapidity. When moving in this way the body of the parasite remains nearly stiff, while the forwardly directed flagellum vibrates with rapid serpentine movements. In a few cases they were found in masses in the proctodæum, but in most cases they occurred in the intestine, swarming and multiplying in the freshly ingested blood. Occasionally they were found passing along the thoracic intestine into the proventriculus. The parasites found in the proventriculus did not differ appreciably either in size or appearance from those found in the digestive tract. By the method suggested by Koch, of compressing the bulb of the proboscis, we succeeded in forcing trypanosomes out from the proboscis, but only in those flies in which the parasites were found in the proventriculus. Of the two types described above, *T. grayi* was the most commonly found, being present in 1·47 per cent. of a total of 3000 flies examined, while *T. tullochii* was found in 0·17 per cent. of flies, and both trypanosomes together in the same fly only three times. When trypanosomes were found in the fly's proventriculus it was more usually *T. tullochii* which was present, while when trypanosomes were found only in the fly's intestine it was more usually *T. grayi* that occurred, but no conclusions can be drawn from this until more flies have been examined.

The object of our experiments on these "fresh fly trypanosomes" was to determine whether one or both of the two types found were or were not developmental stages of *T. gambiense.* As it is now beyond all doubt that *G. palpalis* is the agent which conveys the trypanosome of Sleeping Sickness from an infected to a healthy individual, it would seem most probable at first sight that any trypanosomes found in the bodies of these tsetse-flies caught in a Sleeping Sickness area would be developmental stages of *T. gambiense*. We felt no doubt at the outset of our investigations that these fresh fly trypanosomes were to be identified with *T. gambiense*. Koch* evidently worked on the same assumption, since in his comparison of the supposed developmental stages of *T. brucii* and *T. gambiense* there can be no doubt that he has taken the form which has been called *T. grayi* for a developmental phase of *T. gambiense*. As we proceeded, however, with our investigations we were gradually led to doubt any connection between these "fresh fly trypanosomes" and Sleeping Sickness. In order to determine this point we carried out a number of experiments on flies caught on the island of Kimmi. This island was chosen because it swarmed with these tsetse-flies, of which a high percentage contained trypanosomes, and because it was, and has been for a very long while, quite uninhabited.

Kimmi is a small island of the Sesse group, about two miles long by a mile wide (see map). There is a narrow strip of sandy shore all round it, the remainder of the island being covered with thick undergrowth and forest. On the foreshore are many ambatch trees, where cormorants, other diving birds, and weaver birds are very plentiful. This island is a regular feeding ground for hippopotami and is crossed in all directions by their tracks. Crocodiles are also very numerous. Kimmi is situated about 15 miles from Entebbe and is two miles from Nsadzi Island in the one direction and from Kome Island in the other. For more than a year this island has been quite uninhabited and natives now never visit it. The whole island swarms with tsetse-fly (*G. palpalis*). In spite of the total absence of human beings on Kimmi Island, we found that more than 7 per cent. of the tsetse-flies caught there contained trypanosomes of one or other of the two types mentioned, while only 1·7 per cent. of the flies caught on the main land near Entebbe, a place with a numerous population, among whom Sleeping Sickness is common, contained similar parasites.

Our method of experimenting with these flies was as follows:—Our camp with our apparatus and experimental animals was placed on the neighbouring healthy island of Nsadzi, in a region free from fly and where there is no Sleeping Sickness. A steam-launch was placed at our disposal by the authorities and by means of it batches of flies were brought back from Kimmi, so that we were not obliged to take possibly infected native canoe-men, a class among whom Sleeping Sickness is very common, to this island. These Kimmi flies were divided into batches and each batch assigned to a particular animal (monkey, rat, guinea-pig, or hen) on which the batch

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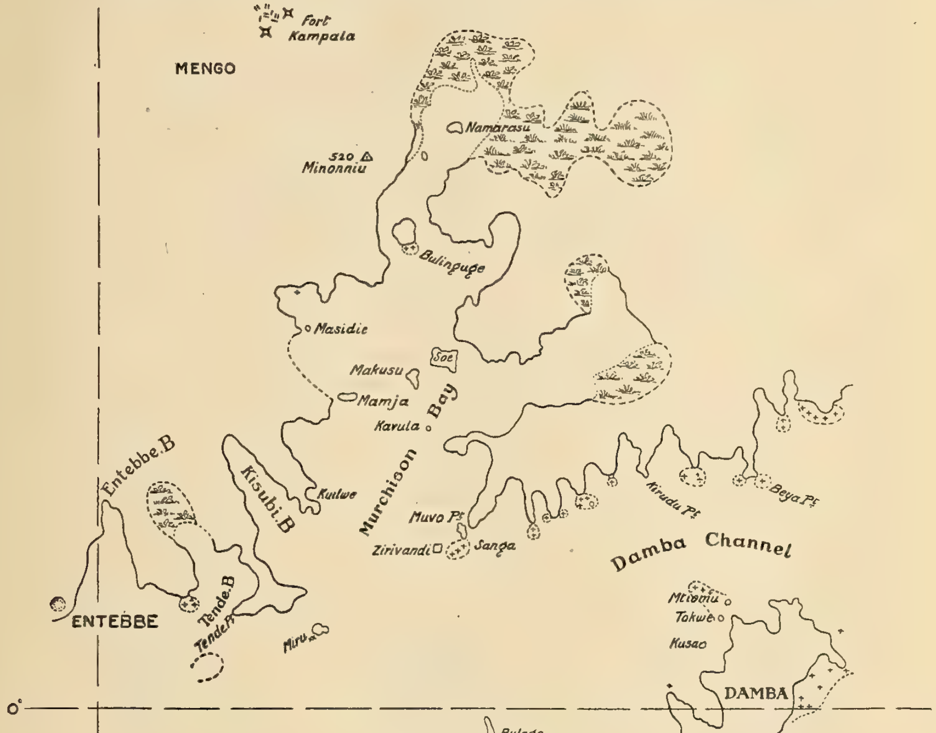
* 'S.-B. k. pr. Akad. Wiss. Berlin, 1905, pp. 958—962.

PART OF THE SESSE ISLANDS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was fed at once, and again repeatedly on successive days. After 12 days or a fortnight of such daily feeding, the flies of each batch were dissected and examined for the trypanosomes which they might contain. In practically every case one or more flies of each batch were found to contain trypanosomes, so that every experimental animal was definitely known to have been fed upon repeatedly by at least one fly containing trypanosomes. Had these trypanosomes therefore been identical with *T. gambiense,* it might have been expected that at least some of these susceptible animals (such as monkeys, guinea-pigs, and rats) would have become infected, *but this did not occur in a single instance.* We thought that *T. grayi* might possibly be a bird-trypanosome, but the negative results of feeding flies containing it on fowls did not bear out this supposition.

In addition to these feeding experiments, we inoculated other experimental animals of the same kinds with the contents of the various parts of the digestive tracts of flies containing these trypanosomes, some from the proventriculus, some from the intestine and some from the proctodæum, *but again in every case the results were negative.*

We are, therefore, now convinced from the results of these numerous experiments, of which a list is given on p. 253, *that the trypanosomes found in the freshly caught tsetse-flies, and referred to by us as* T. grayi *and* T. tullochii, *have nothing to do with Sleeping Sickness and are not developmental stages of* T. gambiense.

It is a matter of regret to us that we have not been able to establish on what vertebrate host, if any, these trypanosomes are parasitic. It seemed at least probable that *T. grayi,* some forms of which greatly resemble *T. johnstoni,* Dutton and Todd* from *Estrelda estrelda,* was taken up by the fly from some of the numerous water birds that haunt the lake-shore. On the other hand, *T. tullochii,* which is very similar in its morphological characters to *T. gambiense,* might similarly be derived from a mammalian host. We may draw attention in this connection to the remarkable manner in which this tsetse-fly haunts the lake-shore. There is nothing in the breeding habits of the fly which should oblige it to frequent the vicinity of water, as in the case of the mosquito. Our experience of flies kept in the laboratory convinced us that a certain amount of moisture is necessary for them, since they died much faster in their cages if not kept over water. It may be supposed, however, that one attraction that the lake-shore exerts upon this voracious blood-sucker is that of food-supply. Along the shores of the lake and on all the small islands are vast numbers of cormorants and other fish-eating birds perched with their wings extended, drying themselves in the sun on the trees, and especially on the ambatch trees, where the flies are found in swarms. These birds might furnish one constant and important source of food. We found in the laboratory that tsetse-flies fed very rapidly on captive fowls, creeping under their wings to bite the poorly protected parts of the skin. On the other hand, when a heap of recently shot water-birds, some of which were hardly dead, were lying on the lake-shore at Kimmi Island, the swarms of tsetse-flies did not attempt to settle on them, although freely biting us and our servants. A second possible source of food supply is furnished by the aquatic animals of the lake-shore, such as the hippopotamus, the otter, the crocodile and the python. We have definite evidence that the fly feeds on the hippopotamus and on the crocodile. Flies were caught in the act of biting a hippopotamus just recently shot, settling chiefly on the ears and nose. We, therefore, made blood-films and had blood-films sent us of as many aquatic birds and animals as possible, including five or six hippopotami. Only in a single case did we find a trypanosome, namely, in a not very well preserved film of crocodile's blood; beyond its large size and general resemblance to other reptilian trypanosomes, it was not possible to make out any details of structure in this parasite. We may mention, however, that the blood of many of the birds contained Halteridia, and that a Hæmogregarine was quite common in the blood of the crocodiles (fig. 1). We also observed that flies in captivity sucked the blood of lizards, chameleons and snakes very freely.

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* Liverpool School of Tropical Medicine, Mem. XI, Pl. 2, fig. 1.

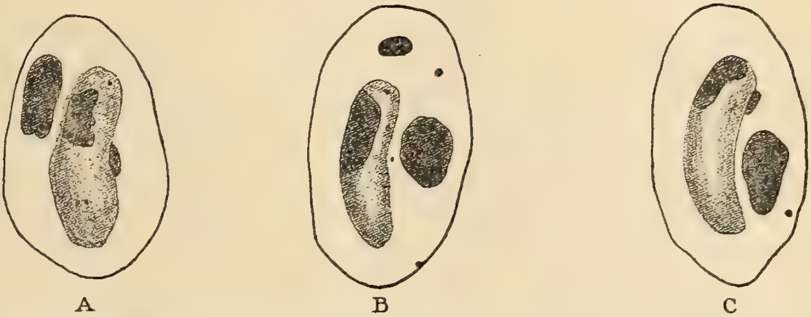


Fig. 1.—Hæmogregarine in the red blood-corpuscles of the crocodile. × 2000.

There are, therefore, two possible sources for the trypanosomes in the freshly caught tsetse-flies. Either they are taken up from some of the numerous animals upon which the fly feeds, or they may be parasites of the fly itself, like *Herpetomonas muscæ-domesticæ* in the house-fly. In this respect it is interesting to note that a small percentage of another common blood-sucking fly in Uganda (*Stomoxys* sp.) contain a species of *Herpetomonas* very similar to that of the common house-fly in Europe. With regard to

G. palpalis we were never able to obtain any definite proof that it fed on anything but blood. It is therefore difficult to understand how a parasite of the tsetse-fly itself could be conveyed from one fly to another except by the hereditary method. We have a single instance to record which certainly suggests hereditary transmission of these trypanosomes. A tsetse-fly was bred in the laboratory in August and was fed for two months on fowls, which were unfortunately also used for feeding our stock of tsetse-flies in our breeding cages. On October 9, the fly was fed on a monkey showing very scanty trypanosomes (*T. gambiense*) in its blood. The next day, 21 hours later, this fly was dissected and found to contain a few scanty *T. gambiense*, one of which is figured on Plate 12, fig. 14, and vast swarms of *T. grayi* (Plate 13, figs. 23 and 28). It is obvious, therefore, that this fly was either infected with *T. grayi* when it emerged from its pupa or that it became infected from one of the fowls which had possibly been infected in its turn by the fresh flies which fed on it. It may be mentioned in this connection that experiments directed towards obtaining flies infected with *T. gambiense* by the hereditary method, that is to say, by breeding from flies fed continually on infected animals, gave no result.

In conclusion, one remarkable experiment of ours may be mentioned. At our camp on Nsadzi, referred to above, we fed a large number of freshly caught Kimmi flies on a goat which we obtained from natives on the island. We then dissected these flies and, to our astonishment, could not find trypanosomes in a single one of some 500 flies which had so fed, whereas in other Kimmi flies, caught at the same time, which had fed on our other experimental animals (monkeys, etc.), trypanosomes were present in the usual proportion. We then prepared some goat's serum and added a drop of it to the contents of a fly's intestine teased out on a slide, which contained *T. grayi* in large numbers. Another drop of this same goat's serum was added to a preparation of *T. gambiense* obtained from an infected rat and the two preparations watched. It was found that in the preparation of *T. grayi* the trypanosomes rapidly became immobile and died off, while the *T. gambiense* remained active. We then tried the same two experiments over again, using human serum instead of the goat's serum, and then found that the trypanosomes were not affected in either case. This result seems to us to furnish an additional means of distinguishing between *T. gambiense* and *T. grayi*.

Appendix I.

Table I.—List of Animals on which Tsetse-flies known to contain Trypanosomes of the two types mentioned have fed. All these animals remained uninfected by this feeding.

Animal.	Number of flies which had fed found to contain trypanosomes.	Class of trypanosome present in fly.	Presence or absence of trypanosomes from fly's proventriculus.
Monkey No. 370	2	*T. grayi.*	
„ No. 391	3	„	
„ No. 369	2	„	
„ No. 397	1	„	Present.
„ No. 335	3	*T. grayi* in two flies. *T. tullochii* in one fly.	Present in one of the former.
„ No. 474	2	*T. grayi* in one and *T. tullochii* in the other.	Present in both.
„ No. 499	4	*T. grayi.*	Absent in all.
„ No. 525	5	„	„
„ No. 553	1	„	Absent.
„ No. 554	4	„	Present in one fly.
„ No. 473	2	*T. grayi* in one and *T. tullochii* in the other.	Present in both.
„ No. 498	4	*T. grayi.*	Absent in all.
„ No. 555	1	„	Absent.
„ No. 556	3	„	Present in two.
„ No. 557	1	*T. grayi* and *T. tullochii* together.	Present.
Guinea-pig, F. F.	8	*T. grayi* in 7. *T. tullochii* in 1.	Present in two.
„ No. 528 ...	5	*T. grayi.*	Absent.
Rat (white), No. 533 ...	1	*T. tullochii.*	Present.
Hen No. 505	4	*T. grayi.*	Present in two.
Hen No. 506	6	*T. grayi* in 5. *T. tullochi* in 1.	Present in three.

Appendix II.—*An Experiment on the Cultivation of* T. gambiense.

By Lieutenant A. C. H. Gray, R.A.M.C., and the late Lieutenant F. M. G. Tulloch, R.A.M.C. (Sleeping Sickness Commission).

Our numerous failures in this direction have been attended by one partial success.

The following method was employed. A tube of agar, prepared according to the formula of McNeal and Novy, was melted and cooled to 60° C. Three times its volume of blood, taken directly from the heart of a dog without defibrination, was added to the agar. The water of condensation was inoculated with a drop of blood from a white rat (No. 513) very rich in trypanosomes. On examining the tube six days later a few living trypanosomes were found, which appeared similar to the forms inoculated. On the

8th and 10th days no trypanosomes were seen in a loopful of fluid withdrawn from the tube. On the 15th day several active trypanosomes were seen in a sample. These trypanosomes were found singly and in groups of three or four. Dividing forms were also seen. These forms were distinctly larger than the trypanosomes originally inoculated, and on measurement were found in some cases to be as long as 54 μ. Besides being longer and broader than the trypanosomes in the blood of the rat, the position of the micro-nucleus was different (Plate 12, fig. 20). In the trypanosomes from the test-tube the micro-nucleus was situated at a considerable distance from the hinder end of the parasite and consequently nearer to the macro-nucleus. These trypanosomes closely resembled certain forms which we have found in the stomach and intestinal tract of tsetse-flies, 24 hours after being allowed to feed on infected monkeys. On the 17th day trypanosomes were still present in about the same numbers, but a few cocci were also found in the tube. Up to the 20th day trypanosomes were still found, but were sluggish in their movements and became fewer in number as the cocci increased. After this date the growth of cocci became profuse and the trypanosomes died off. Up to the present (seven days) no trypanosomes have been found in sub-cultures made from this tube, although the latter are free from bacteria.

As multiplication had commenced in the original tube, it is reasonable to expect that a successful culture would soon have resulted if it had not become contaminated by cocci.

The resemblance of the newly formed trypanosomes to forms seen in tsetse-flies after feeding on infected animals is of interest.

APPENDIX III.—*Some Notes on a* Herpetomonas *found in the Alimentary Tract of* Stomoxys (calcitrans ?) *in Uganda.*

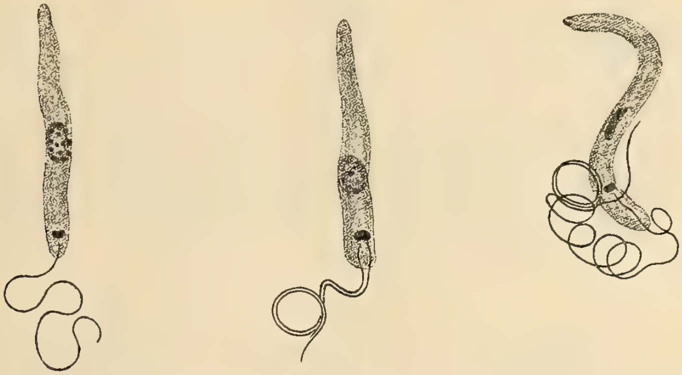
By A. C. H. Gray, M.B., Lt. R.A.M.C.

In the course of examining the contents of the alimentary tract of some specimens of *Stomoxys* (*calcitrans?*), which had previously been allowed to feed themselves on a monkey infected with the trypanosome of Sleeping Sickness, I found a species of *Herpetomonas* in the alimentary tract of three flies out of a total number of 280 examined.

In its movements, size, and general appearance, the flagellate seemed to closely resemble *H. muscæ-domesticæ* of the common house-fly.

In two flies this parasite was present in very large numbers. Those two flies were full of blood from the monkey they had fed on 24 hours previously, and in this blood practically unaltered *T. gambiense* were present in scanty numbers. In the third fly this *Herpetomonas* was present in very scanty numbers and no trace of recently ingested blood could be found in it.

Films, fixed in osmic acid and stained with Borrel blue and eosin, showed that the commonest type of this parasite measures from 35 to 50 μ (figs. 2—4).

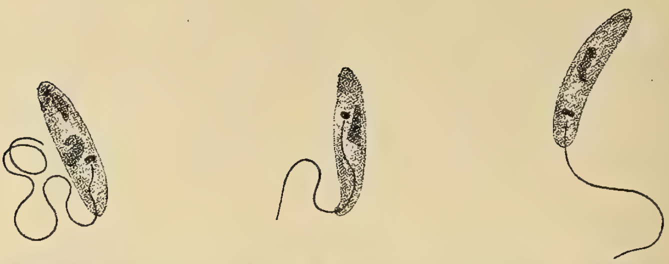


Figs. 2—4.—*Herpetomonas* from the gut of *Stomoxys* (*calcitrans?*); fig. 2, common form with single flagellum, and with nucleus broken up into separate masses; fig. 3, commonest form, with double flagellum; fig. 4, form with compressed nucleus and very long flagellum.

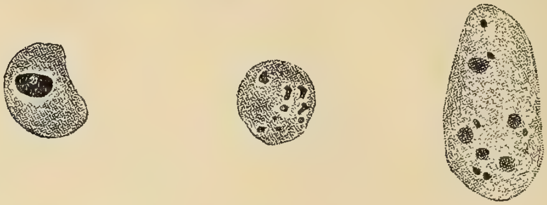
The body of the parasite is cylindrical, with a rounded anterior and more pointed posterior extremity. The protoplasm of the body stains rather deeply. A large rounded nucleus is placed at the centre of the body of the parasite. The chromatic substance of the nucleus is sometimes seen to be broken up into granules (chromosomes), apparently 14 in number, contrasting in this respect with *H. muscæ-domesticæ,* in the nucleus of which eight chromosomes are present (Prowazek). The blepharoplast is oval or kidney-shaped, of a large size and stains deeply. It is placed close to the origin of the flagella and to the anterior rounded extremity of the body of the parasite. The double flagellum arises close to the blepharoplast and may reach an enormous length in some individuals (fig. 4).

Besides these large forms, smaller individuals are present (figs. 5, 6, 7). The bodies of these parasites stain more faintly than the above and are often curved. The nucleus is more compressed. The blepharoplast is smaller and situated at a greater distance from the anterior extremity of the body. The single flagellum arises close to the blepharoplast and consequently has a somewhat longer course through the body of the parasite. It emerges as a short, thick, free flagellum.

Both these forms commonly undergo longitudinal division. In some cases the nucleus apparently divides before the blepharoplast.



FIGS. 5—7.—*Herpetomonas* from gut of *Stomoxys;* fig. 5, small form with dividing nucleus; fig. 6, small form showing the posterior position of the blepharoplast, and long intracellular course of the flagellum; fig. 7, small form, ordinary type.



FIGS. 8—10.—*Herpetomonas* from gut of *Stomoxys*, non-flagellated forms; fig. 8, mass of blue-staining protoplasm containing one large chromatin body; figs. 9 and 10, masses of protoplasm containing paired chromatin bodies.

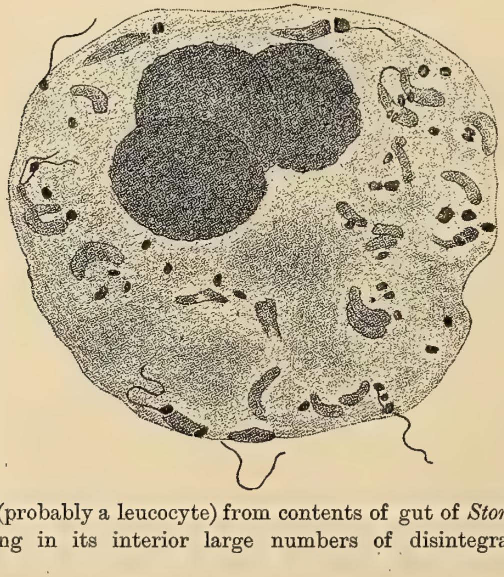


FIG. 11.—Large cell (probably a leucocyte) from contents of gut of *Stomoxys* (*calcitrans ?*), containing in its interior large numbers of disintegrated *Herpetomona* forms.

In the third fly non-flagellated forms were found to occur (figs. 8, 9). Masses of blue-staining protoplasm containing chromatin bodies in pairs (fig. 10) were also rarely found in this fly. Every such pair of chromatin bodies consisted of a larger and a smaller separate portion. The larger portion is circular and more faintly staining, the smaller is oval and more deeply staining. These paired masses of chromatin suggest a form analogous to Leishman-bodies.

On several occasions, in the first two flies, large cells (leucocytes ?) were found containing in their interior the broken-up remains of large numbers of the *Herpetomonas* (fig. 11).

The figures illustrating these notes are all × 2000 and drawn with the camera lucida from slides fixed in osmic acid and stained with Borrel blue and eosin.

DESCRIPTION OF PLATES.

Plate 12.—*Trypanosoma gambiense*, figs. 1—14, forms from the gut of the tsetse-fly (*Glossina palpalis*) one day after infection, (*i.e.* about 24 hours after being taken up by the fly. Fig. 15, two days (48 hours) in the fly. Figs. 16—19, forms from the blood of a monkey (*Cercopithecus* sp.) infected with the injection of cerebro-spinal fluid from a Sleeping Sickness patient. Fig. 20, culture form from blood of infected rat, 15th day. All except figs. 14 and 20 preserved wet with osmic vapour stain, Leishman or Geimsa. × 2000.

Figs. 1 and 2, male form with compressed nucleus. Fig. 3, male form with rounded nucleus. Figs. 4 and 5, similar forms with chromatin being given off from the nucleus. Fig. 6, male form dividing, chromatin being given off from both the daughter nuclei. Figs. 7—14, female forms, figs. 7 and 12 dividing. Fig. 15, indifferent form. Fig. 16, male form. Fig. 17, female form, very scarce (only one other was found). Figs. 18 and 19, indifferent forms which in the fly become female ; note the short, free flagellum. Fig. 20, female form from culture tube, 15th day.

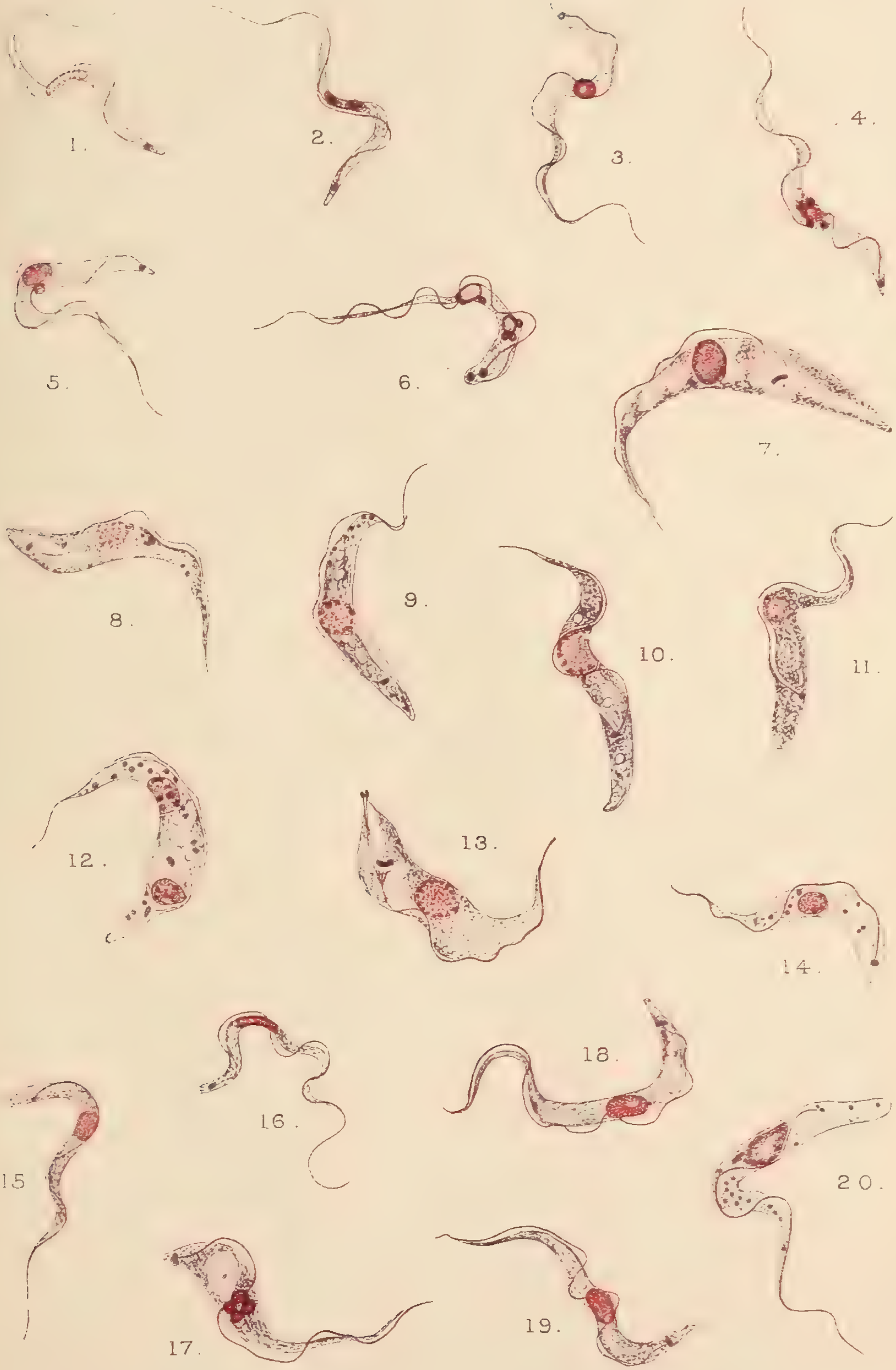
Plate 13.—*Trypanosoma grayi*, figs. 21—30, from the gut of the tsetse-fly (*Glossina palpalis*). The flies from which these trypanosomes were obtained had been fed regularly on the blood of neutral monkeys and were dissected after about 10 days of such feeding In all cases the whole gut of the fly swarmed with thousands of similar trypanosomes. Figs. 31 and 32, small forms ; flies dissected 24 hours after their first feed of blood generally contained forms such as these in very great abundance, many dividing forms were also present, larger forms, as above, were rare. Figs. 33—35, trypanosomes from fresh-caught tsetse flies, which had not fed on blood for a long while. In such flies, parasites were never very numerous and the types present were all of a large size. Figs. 36—40, various other types from flies which had fed on blood. Fixed in alcohol, stained with Leishman or Giemsa. × 2000.

Figs. 21 and 22, male forms with compressed nucleus and long free flagellum. Figs. 23, 24 and 25, female forms. Posterior extremity thickened, short free flagellum. Figs. 26, 27 and 28, stages of division showing the unequal size of the two resulting individuals. Fig. 29, division of a small form into two more or less equal-sized individuals. Fig. 30, aberrant dividing form, in this case the nucleus has already divided, whereas the blepharoplast has not yet done so. Figs. 31 and 32, young forms resulting from the unequal division of a large female form. Fig. 33, very long male form. Fig. 34, very large female form. These two forms occur in flies which have not fed on blood for a long time. Fig. 35, very long form, with nucleus consisting of eight chromosomes. Figs. 36—40 are all from the same fly. Figs. 36, 37, and 38 show the nucleus broken up into separate chromatic granules, and the varying position of the blepharoplast. Fig. 39, young form showing regular division of the nucleus into eight chromosomes. Fig. 40, young form showing a separate mass of chromatin posterior to the nucleus.

Plate 14.—*Trypanosoma grayi*, figs. 41—52; *Trypanosoma tullochii*, figs. 53—60; all from the gut of the tsetse fly (*Glossina palpalis*). Fig. 41, very large female form, undergoing unequal division, from a fly which had not fed on blood. Fig. 42, rosette-like mass of similar young forms. Figs. 43—51 are all taken from the same fly, and show the various steps in the formation of circular non-flagellated forms from a common type, fig. 43. Figs. 44 and 45 show the flagellum becoming wrapped round the body of the parasite. Figs. 46, 47, and 48 show progressive stages in the absorption of the flagellum. Figs. 49, 50, and 51 are different types of the resulting non-flagellated bodies. Fig. 52, an uncommon type of parasite, with very large blepharoplast, and showing much chromatic granulation, from a fly which had not fed on blood.

Figs. 53—60, *Trypanosoma tullochii*. Fig. 53, small form, with minute circular blepharoplast and small vacuole. Fig. 54, larger form of a similar type. Fig. 55, small form showing the flagellum arising from a small granule of chromatin close to the blepharoplast. Fig. 56, large form. Figs. 57 and 58, large forms of trypanosome found in the proventriculus of an infected fly. Fig. 59, dividing form, common type. Fig. 60, dividing form, rare type showing division into three. Figs. 53—60 are all from the same fly. Figs. 57 and 58 are from the proventriculus, while the remainder are from the gut of the fly (*Glossina palpalis*).

Minchin, Gray & Tulloch. *Roy. Soc. Proc. B. Vol. 78. Pl. 12.*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E. A. M. and A. C. H. G. del. Huth, Lith^r London.

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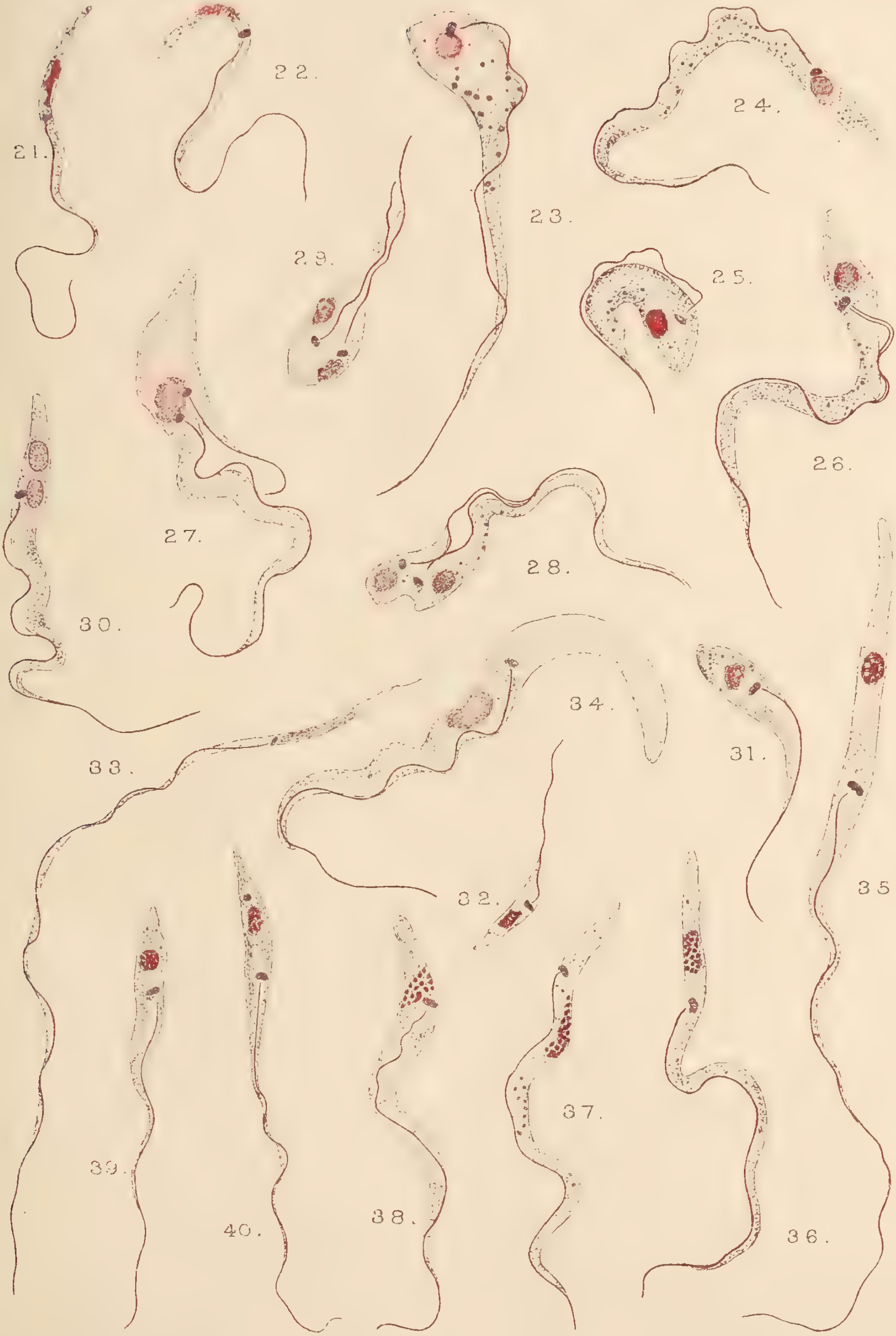
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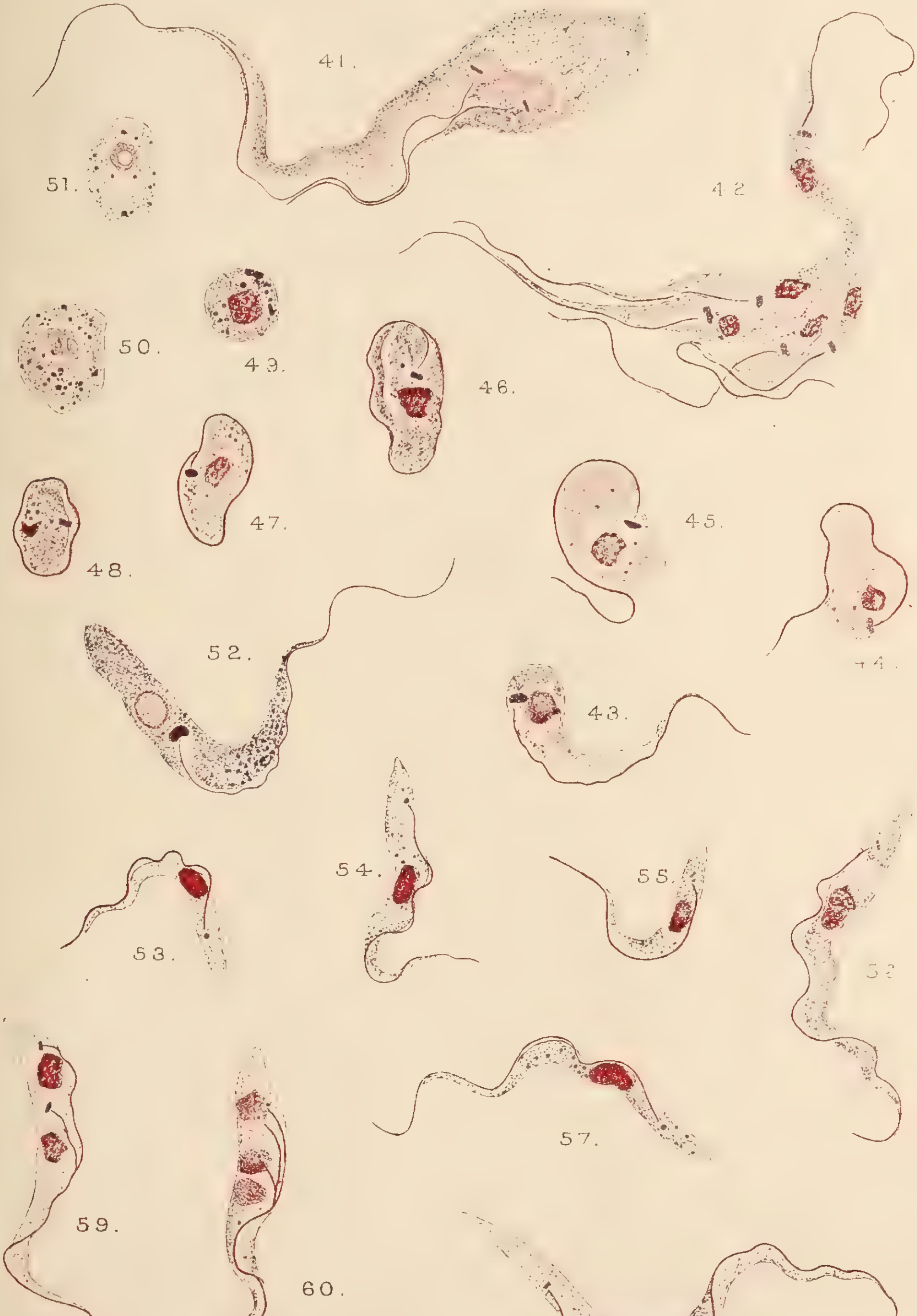
TRYPANOSOMA GRAYI × 2000.

Minchin, Gray & Tulloch. *Roy. Soc. Proc. B. Vol. 78. Pl. 11.*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TRYPANOSOMA GRAYI AND T. TULLOCHII × 2000.

Regeneration of Nerves.

By F. W. Mott, M.D., F.R.S., W. D. Halliburton, M.D., F.R.S., and Arthur Edmunds, M.S., B.Sc., F.R.C.S.

(Received June 12,—Read June 28, 1906.)

[Plate 15.]

In 1901 two of us published a paper on Nerve Degeneration,* a subject which it is almost impossible to study without taking into account the closely-related subject of nerve regeneration. From the microscopic study of the distal portions of divided nerve trunks we arrived at the conclusion that the activity of the neurilemmal cells has some relation to the development of the new nerve-fibres. At an early stage in degeneration they multiply; later they participate with phagocytes in the removal of the broken-up myelin droplets; subsequently they elongate, and, becoming connected end to end, lead to the formation of what some term embryonic nerve-fibres. These three stages are illustrated by some of the microphotographs published in the paper alluded to. (See especially figs. 22, 25, and 27.)

We were, however, extremely doubtful whether this appearance really indicated the formation of real nerve-fibres capable of conducting impulses, and felt that such incomplete observations could not be considered as serious objections to the view of those who, from Waller onwards, have taught that the axis cylinder is the branch of a nerve-cell which grows towards the periphery.

In a preliminary communication† which we published two years ago we called special attention to the work of Howell and Huber‡ on the subject. These observers, who employed both histological and experimental methods of observation, and who noted, as all other writers before and since have done, the neurilemmal activity, arrived at the conclusion that although these peripheral structures are active in preparing the scaffolding, the axis cylinder, which is the essential portion of a nerve-fibre, has an exclusively central origin. We further stated that the more work we have done on the subject the more have we become convinced that this view is the correct one.

The purpose of this paper is to state more fully the evidence that has led us to this conclusion.

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* "The Chemistry of Nerve-Degeneration," by F. W. Mott and W. D. Halliburton, 'Phil. Trans.,' B, vol. 194, pp. 437—466, 1901.

† 'Proceedings of the Physiol. Soc.,' March 19, 1904; 'Journal of Physiology,' vol. 31.

‡ 'Journ. of Physiology,' vol. 13, p. 333 1892); vol. 14, p. 183 (1893).

Since we commenced our work, the subject has risen into considerable prominence, and numerous papers have been published on the question. Some investigators, like Purves Stewart, and Ballance,* in this country, and Bethe† in Germany, have advanced the view that the new nerve-fibres have a peripheral origin, while others, like Langley and Anderson,‡ and S. Ramon Cajal,§ have defended the older Wallerian doctrine. In Cajal's paper a very complete bibliography of the large number of investigations devoted to the enquiry is also given.

We ventured to suggest in the preliminary notice of our work that the manifest activity of the neurilemmal cells is related in some degree, probably nutritionally, to the successful repair of a divided nerve, for in situations like the central nervous system, where the neurilemma does not exist, not only is the removal of degenerated myelin a very slow process, but, as is well known, regeneration does not occur. When the nerve is regenerated, and conducts impulses, the elongating strands of neurilemmal cells are seen to be situated outside the new axis cylinder, and in longitudinal view the latter is thus frequently concealed; but on transverse section the axis cylinder is seen to be quite distinct and separate within this sheath, although the neurilemmal nuclei retain an abnormal thickness for some time.||

The idea that the sheath is nutritive has been advanced in a somewhat modified form more recently by Graham Kerr.¶ He points out that there are three main views regarding the development of motor nerves in vertebrata:—

1. Each nerve-fibre develops as an independent outgrowth from a ganglion cell, which gradually grows outwards and finally becomes united to its special muscle. This view is associated especially with the name of His, and is favoured by the majority of embryologists.

2. The nerve trunk is regarded as multicellular in origin, and consists at first of a chain of cells, in the substance of which the nerve-fibres are developed later as fine strands passing continuously from one cell body to

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* 'The Healing of Nerves,' Macmillan and Co., 1901.

† 'Neurol. Centralbl.,' January, 1902, p. 60; 'Allgemeine Anatomie und Physiologie des Nervensystems,' Leipzig, 1903.

‡ 'Journ. of Physiology,' vol. 31, p. 418 (1904).

§ "Mecanismo de la regeneración de los Nerviós," 'Trabajos del Laboratio de investigaciones biologicas de la Universidad de Madrid,' vol. 4, pp. 119—210 (1905); "Mécanisme de la Régénération du nerf," 'Compt. rend. Soc. de Biol.,' vol. 59, p. 420; "Critique de la théorie de l'autorégénération," *ibid.*, p. 422.

|| This is well illustrated in fig. 30, p. 464, of our 'Phil. Trans.' paper.

¶ "On some Points in the Early Development of Motor Nerve Trunks and Myotomes in Lepidosiren," by J. Graham Kerr, 'Roy. Soc. Edin. Trans.,' vol. 41, pp. 121—128 (1904).

another. The elements forming the original chain are regarded as ectodermal elements which have wandered out from the spinal cord rudiments. The sheath is derived from those parts of the original cell chains which retain their protoplasmic character.

3. The nerve trunk is not a secondarily formed bridge between the spinal cord and the peripheral organ, but exists from the first, and in subsequent development it merely undergoes elaboration and increase of length as the distance between the spinal cord and the periphery increases with the increasing size of the animal.

His study of the development of motor nerves in the fish Lepidosiren leads Kerr to adopt the third view as regards the nerve trunk itself, and the second view as regards its sheath. In early stages the motor trunk is naked, but later masses of mesenchymatous protoplasm, laden with yolk, become applied to the nerve trunk, at first over only a small portion of its length; these masses gradually spread over the whole trunk, from which they are clearly distinguishable by staining reactions. As development goes on, the yolk in these masses is used up, their protoplasm becomes less and less conspicuous, and eventually is only to be detected in the immediate vicinity of the nuclei. At first the central protoplasmic strand in each fibre is simply granular, but later the passage of impulses finds expression in the marking out of definite fibrillar tracks, some undifferentiated protoplasm remaining as the inter-fibrillar substance.

It is pretty clear that the great function of the sheath is as a nutritive organ; its protoplasm is at first laden with yolk, and this is gradually used up as the nerve trunk develops within it.

That the main function of nuclei, apart from reproduction, is to control cytoplasmic metabolism is well recognised, and the nuclei of the sheath are able to exercise this control over the active metabolism of the developing nerve trunk, which is destitute of nuclei of its own. Connected with this relation is, no doubt, the active multiplication of these nuclei observed in early stages of nerve regeneration. In such regeneration it may well be that the protoplasmic matrix of the nerve simply repeats the process of its original development, increasing in size and then developing nerve fibrils within itself.

On this view the process which takes place in the peripheral segment of a cut nerve would be somewhat as follows:—

The fibrils, no longer subject to the stimulus of passing nerve impulses, revert to their protoplasmic condition, and the sheath becomes highly active: it increases in thickness, and its nuclei multiply; its protoplasm digests the remains of the medullary sheath. Supplied with nourishment by the activities of the surrounding sheath, the protoplasm behaves just as it does in ontogenetic development; it grows—probably slowly—and so gaps are bridged over; as soon as it becomes continuous, nerve impulses begin to play backwards and forwards in its substance and cause again a differentiation into fibrils. As part of the impulse tracks persist at the central stumps of the fibrils, the regenerated parts of the fibrils will naturally develop in exact continuity with these.

We have reproduced Professor Graham Kerr's views at some length because they support, in an entirely independent manner, our own views on the nutritive properties of the nerve sheath. Whether, however, such nutritive control is able to act so far as to produce actual fibrillæ we are extremely doubtful. Graham Kerr admits that in a degenerated and regenerating fibre the protoplasmic strand can only be demonstrated with extreme difficulty, and he further admits the necessity of union with the central stump to ensure functional regeneration. In our own experiments, as will be more fully described later, we have never seen anything of the nature of an axis cylinder or neuro-fibrillæ in the peripheral segment of a divided nerve, provided connection with the central end is successfully prevented.

Graham Kerr further emphasises the fact that his observations apply to one animal only, and that he does not wish to draw general conclusions from them as to what occurs in other vertebrates. He, however, points out that Bethe's observations on the chick, although apparently supporting the cell-chain view, are in no way irreconcilable with the observations he chronicles. With regard to the view he advances that the original nerve trunk exists from the first as a link which stretches in length with the growth of the animal, it appears to us that it is incapable of explaining how a gap an inch or so long can be bridged across when one excises, as we have done in some of our experiments, a portion of nerve of that length. The union that occurs, often very rapidly, even when the ends are not sutured together, must be due to growth of nerve fibres from the central to the peripheral stump or in the reverse direction.

In a paper published by the veteran histologist, von Kölliker,* shortly before his death, the theory that regeneration can occur in the peripheral end of a cut nerve without connection or control from the central end is very vigorously criticised. Kölliker himself was instrumental many years ago in putting forward the view that the nerve sheath, known as the neurilemma, is a secondary formation in the mesoblast into which the axons penetrate, and in his later years became an exponent of the neuron theory. It is mainly

* 'Anat. Anzeiger,' vol. 25, p. 1 (1904).

from these two points of view that he has attacked Bethe's position, and it certainly appears on *a priori* grounds exceedingly improbable that mesoblastic cells should be capable of giving rise to such a highly specialised structure as an axis cylinder, which so far as is known is exclusively epiblastic in origin.

We may, however, now pass from the region of speculation to consider briefly a few typical researches dealing with the facts of regeneration, before describing our own observations.

The propositions maintained by Waller,* namely, the integrity of the central end of a cut nerve, the degeneration of the peripheral segment, and its regeneration by fibres growing out from the central stump, were questioned a few years later by Vulpian and Philippeaux.† These observers cut nerves in various, but mainly, young animals, excising long portions to prevent reunion with the central end. Some months later they were surprised to find that the peripheral ends had regenerated and were excitable; to this phenomenon they gave the name of "autogenetic regeneration." These results were regarded with great scepticism by most writers,‡ until, in 1874, Vulpian§ repeated his experiments, and obtained the same results as before. This time, however, Vulpian put forward a new explanation and, in fact, accepted the Wallerian doctrine, because he found that connection with the central nervous system had been re-established by means of fibres growing into the peripheral stump from other nerves in the neighbourhood. He had also, with Philippeaux, observed regeneration in portions of nerve trunks transplanted under the skin of the abdomen, and explained this in 1874 in the same way.

In the revival of the controversy that has taken place within the last few years the position of the disputants has been almost exactly the same as that occupied by Waller and Vulpian more than half a century ago.

Ballance and Purves Stewart‖ hold the view that the new nerve-fibres have a peripheral origin, but rely exclusively on histological evidence; one method they employed was Golgi's, which can hardly be considered for this purpose a trustworthy one. It is well known that black streaks are produced by this method by structures which are not nervous at all. A strand that looks like a nerve-fibre is not really such unless it can be experimentally shown to be both excitable and capable of conducting nerve impulses. In the absence of any such proof we regard it as probable that these observers have mistaken for nerve-fibres the chains of neurilemmal cells which undoubtedly form in the peripheral portions of cut nerves.

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* "Nouvelle méthode anatomique pour l'investigation du système nerveux," 'Comptes Rendus,' 1852; "Expériences sur les sections des nerfs," 'Gazette Médicale,' 1856.

† "Note sur les expériences démontrant que les nerfs séparés des centres nerveux . . .," etc., 'Comptes Rendus,' 1859.

‡ See, for instance, Ranvier, 'Leçons sur l'histologie du Système Nerveux,' 1873, pp. 158, 186, and 190.

§ 'Archives de Physiologie,' 1874, p. 704.

‖ *Loc. cit.*

Bethe* is at present the most prominent supporter of the autogenetic theory, and his observations on the return of histological structure have been coupled with experimental testing. He finds that the peripheral ends of cut nerves are in young animals excitable without union with the central end. He does not, however, exclude the fallacy which underlay the old experiments of Vulpian and Philippeaux, and which has more recently been pointed out by Langley and Anderson.† These observers showed that, in spite of any obvious connecting strand with the central end, new nerve-fibres find their way often by devious channels into the peripheral stump from nerves in skin and muscle cut through in the operation. Bethe, in fact, by burying the peripheral end of a nerve in the neighbouring musculature in order to prevent reunion with the central stump, provides an excellent means for the muscular nerves to carry out that union with the central nervous system which it was his object to avoid.‡

At the commencement of their work Langley and Anderson thought they had obtained evidence of purely peripheral regeneration, and it was not until they carried out careful dissections that they convinced themselves that union with the central nervous system had occurred in the manner just mentioned. They, therefore, once more cut the central end of the nerve, and of any other nerves which might possibly have established a connection with the peripheral segment of the nerve they had under observation. The second operation would cause degeneration of the fibres which had established a central connection and leave intact any fibres which had regenerated autogenetically. Even this was not always sufficient to prevent reunion once again of the peripheral end with the central nervous system. But in cases of success no autogenetically formed nerve-fibres were found; eight to twelve days after the second operation, no sound medullated fibres were found in the peripheral end, and the nerve was quite inexcitable. These experiments, like Bethe's, were mostly performed upon young animals (kittens and rabbits), and in some, after a period of nearly two years, no trace of autogenetically-formed new nerve-fibres could be detected.

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* *Loc. cit.*

† *Loc. cit.*

‡ This view is also taken by Münzer, 'Neurol. Centralbl.,' December 1, 1902 (p. 1090), and January, 1903 (p. 63).

All the medullated nerve-fibres found in the peripheral end of a cut nerve, which had been sutured to the central end, degenerated when the central end was again cut nearer the spinal cord; in other words, all the nerve-fibres were fibres which were connected with the central nervous system. If the regeneration had been autogenetic, this would mean that every one of them had become united with the end of a nerve-fibre in the central stump; on the autogenetic theory this is highly unlikely, whereas it is quite simply accounted for on Wallerian lines. If the number of medullated fibres in the peripheral end was small, then the connection with the central end was found to be slight. If no connection occurred, then medullated nerve-fibres were entirely absent. Bethe admits a variability in the number of medullated fibres, and this again, though easily explicable on the view that such fibres come from the central end, is not accounted for at all by the autogenetic theory.

Langley and Anderson further point out that Bethe admits age to be an important factor in the case; in adult animals he has little or no proof of autogenetic regeneration. This is a very weak point, for surely the difference is due to the greater ease with which reunion with central nerves occurs in young and growing animals.

Lugano* objects to Bethe's views on much the same lines as Langley and Anderson.

Cajal,† from the histological point of view, has made a very complete rejoinder to Bethe's work. He has also controlled his results by noting the effects of stimulating the nerves. We are unfortunately not able to read the full account of his work in the original Spanish, but the numerous illustrations he gives remind us very forcibly of what we have seen in our own microscopical work. We have, therefore, to rely on the brief summary he has published in French. By the help of his new silver method he comes to the conclusion that the new formation of nerve-fibres in the peripheral stump is exclusively due to growth from the central end. He describes the long and often contorted course of these growing fibres in the swelling at the cut central end, and shows that they ultimately reach their goal, the peripheral stump, in time and in spite of all hindrances. The greater the obstacles interposed, the later does the union and consequent regeneration in the peripheral end occur. He also calls attention to the olive-shaped swelling at the free end of each growing axis cylinder. These are also figured by Marinesco and Minea.‡

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* 'Riv. di Patologia nervosa e mentale,' November, 1904, p. 550.

† *Loc. cit.*

‡ "La loi de Waller, et la régénérescence autogène," by M. G. Marinesco and J. Minea, 'Revista Stiintelor Medicale,' No. 5, September, 1905, Bucharest.

The view taken by the last named observers seems, like Graham Kerr's, to be somewhat of the nature of a compromise.

They point out that all authors are agreed as to the activity and multiplication of the neurilemmal cells in the peripheral segment of a cut nerve, and that these unite together into long strands, but if the central end is compared with the peripheral the activity in the former is much greater and the formation of nerve-fibres is there rapid; these grow in length, assume a contorted course, become myelinated, and an axis cylinder is well defined. In the peripheral end the process is much slower, excitability never returns if union with the central end is prevented, and no real differentiation of axis and sheaths takes place. If the ends are united, then restoration of the peripheral end is more rapid.

They point out that this is due to the fibres at the central end remaining in communication with their cells of origin and so are under their influence. The cutting of the nerve has suppressed in the peripheral end all functional acts, and in consequence all nutritive acts depending on the nervous centres cease also. In the central end, on the other hand, the internal work elaborated by the cells still reacts on the fibres and favours their nutrition and growth.

By the help of Cajal's silver method they describe and figure rows of granules formed in the peripheral segment, especially in the neighbourhood of the neurilemmal nuclei and independently of central union, and believe that these unite to form neuro-fibrillæ which, however, do not become functional until activated by union with the central fibres.* Cajal, however, criticises this part of their observations and conclusions, and does not believe that the striations described are real neuro-fibrils.

In a sense, therefore, they believe in autogenetic regeneration; in fact they regard it as the only form of regeneration, and that the difference between what is seen at the central and peripheral ends is one of degree only, being more active and efficient at the former situation.

They, however, part company from Bethe, for they regard the functional activity of the central nervous system as being absolutely indispensable for the return of function in the nerve-fibres and also for their complete histological restoration. They consider that the nerve cell and its axis cylinder constitute a functional unit, and regard the mere fact that the latter undergoes degeneration when cut off from its cell of origin as sufficient evidence of that.

Perhaps the most steadfast upholders of the theory that peripheral nerves regenerate independently of the central nervous system have been certain surgeons.* The statements made by clinical observers that in man (including adults, in spite of Bethe) sensation rapidly returns after freshening up and suturing together the ends of a nerve which has been divided a long time previously would be very valuable evidence in favour of the "peripheral theory" if it was entirely trustworthy. Since commencing this enquiry, one such case recently in King's College Hospital was carefully observed by one of us (A. E.) and it throws useful light on the subject. The case was not one of actual division, but compression, which comes to much the same thing. Two thick silver wires put on to secure the ends of a fractured humerus had included the musculo-spiral nerve. The operation consisted in removing the wires and freeing the nerve from the scar. A short time afterwards the man stated he was again able to feel, but these sensations rapidly subsided and sensation did not really return until months later. The preliminary sensation was doubtless subjective; the irritation of the nerve-fibres in the scar lasted some hours, and the sensations so produced were referred by the patient's mind to the original terminals of the fibres.

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* Similar appearances were also described by Büngner, 'Ziegler's Beiträge,' vol. 10, p. 321 (1891).

At the meeting of the British Medical Association in Oxford in 1904, Dr. Kennedy,† of Glasgow, brought forward a number of cases in support of the autogenetic theory. Here again, the genuineness of the early recovery is doubtful; in spite of sensitiveness to such tests as needle pricks there is usually absolute anæsthesia to the far more delicate test of stroking the hairs over the affected region until quite late dates. Head and Sherren‡ have also recorded a number of surgical cases, but they found no evidence of early recovery; they showed by very careful work that there is considerable difficulty in localising the stimulus so that it should not affect the hyperæsthetic marginal zone of the anæsthetic region, and also that the deep sensibility of the subjacent parts, such as would be excited by needle pricks, is entirely independent of cutaneous sensation. The fibres subserving this form of sensation run mainly with the motor nerves and are not destroyed by division of all the nerves to the skin. Neglect to recognise these facts will no doubt explain the results recorded by Kennedy and those who have published similar cases.

The difficulty of obtaining absolutely trustworthy evidence from patients themselves induced Head§ to combine with his experiments on animals and his observations on patients an experiment on one of the sensory nerves of his own arm. This experiment gave an opportunity for the study of the phenomena by a trained observer upon himself. He certainly experienced no early return of function, and the date at which sensation did come back coincides closely with the dates obtained in animals by physiologists for the reappearance of new fibres. He has further made the interesting suggestion that the first kind of sensation to return and which is of a vague nature (termed *protopathic*) is associated with the activity of the fine medullated nerve-fibres which replace the degenerated ones at an early stage. Return of protopathic sensibility begins about the eightieth day. The more elaborate sensations and power to accurately localise them return at a much later date, and this *epicritic sensibility*, as Head terms it, is usually not perfect until many months, or even a few years, after the regeneration started. By this time, as was shown by experiments on animals, the fine nerve-fibres which subserve protopathic sensation are largely admixed with a later growth of larger nerve-fibres, and he believes epicritic sensation is subserved by these. He also postulates that the three kinds of sensation (deep, protopathic, and epicritic) are related to different kinds of end organs in the peripheral structures. It is not, however, necessary to enter more fully into these results, for we have made no special study of the varieties of sensation, nor have we in our experiments on animals kept them alive for a sufficiently long period to enable us to see the fibres formed at very late stages.

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* See, for instance, Bowlby, 'Lancet,' 1902, vol. 2, pp. 129, 197.

† 'Brit. Med. Journ.,' 1904, vol. 2, p. 729; see also R. Kennedy, 'Phil. Trans.,' B, pp. 188, 257 (1897).

‡ 'Brain,' vol. 28, p. 117 (1905).

§ Head, Rivers, and Sherren, "The Afferent Nervous System from a New Aspect," Brain,' vol. 28, p. 99 (1905).

After this introduction we propose to pass now to the consideration of our own experiments. After the very conclusive researches of such investigators as Langley and Anderson and of Cajal, it may seem rather a work of supererogation to describe any more experiments which tell against the autogenetic theory. We will only plead that every piece of confirmatory evidence is useful if the upholders of that theory are to be convinced they are wrong, and, moreover, in some directions our experiments are new, and approach the problem in rather a different way from that followed by other workers.

We will describe them under the following heads:—

(1) Experiments in which union of central and peripheral ends was prevented.
(2) Experiments on transplanted pieces of nerve.
(3) Experiments on the degeneration of regenerated fibres.
(4) Experiments on the rate of medullation in regenerating nerves.
(5) Experiments on the influence of stimulus on regeneration.

In carrying out this work we should mention that the experiments on animals (monkeys and cats) have been carried out at King's College, London, by two of us (W. D. H. and A. E.), using anæsthetics (ether and chloroform) during all cutting or stimulating operations and with strict antiseptic precautions. The histological portion of the research has been carried out by the third (F. W. M.).

(1) *Experiments in which Union of Central and Peripheral Ends was Prevented.*

In some of our previous work we noticed that the excision of any inch or so of nerve was entirely inefficient to prevent union of the two ends together; we were later aware, from a preliminary communication made by Langley and Anderson* of their results, that the peripheral end is often invaded by new nerve-fibres from the nerves in skin and muscle divided in the operation.

We, therefore, made extremely small incisions, and the parts were separated from the nerve trunks by the handle of the scalpel, and with as little cutting as possible. In cats one incision over the buttock allowed us to divide the sciatic nerve high up; another in the ham enabled us to divide the two popliteal nerves. The intervening portion of the sciatic nerve, about 4 or 5 inches long, could then be easily pulled out. Additional security to prevent union with central fibres was in some cases obtained by enclosing the upper end of each popliteal nerve in closed caps made out of small sterilised drainage tubes about half an inch long. A period of 100 to 150 days was then allowed to elapse in order that if regeneration was going to occur in the peripheral segments of the nerve it might have an opportunity of doing so. At the end of this time the animal was again anæsthetised and the nerves tested by electrical stimulation. In all cases they were entirely inexcitable to strong faradic currents, and the wasted muscles supplied by these nerves had also largely lost their power of response to this form of stimulation. To the naked eye the nerves were pale. The animals were then killed and the nerves subjected to microscopical investigation, but they were found to show no trace of regeneration. The chains of neurilemmal cells, without a sign of either axis cylinder or medullary sheath, were all that could be seen, and in those cases of later date where the nerves had been placed in tubes it was very difficult to recognise any nervous structure whatever.

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* 'Proc. Physiol. Soc.,' December 13, 1902; 'Journ. of Physiol.,' vol. 29, p. ii.

(2) *Experiments on Transplanted Pieces of Nerve.*

We have already seen that Vulpian and Philippeaux transplanted pieces of nerve under the skin of the abdomen, and observed in them what they at first thought was evidence of auto-regeneration, a conclusion subsequently withdrawn by Vulpian. Similar experiments, recently published by Kennedy,* are regarded by him as proving absolutely conclusively the autogenetic origin of nerve-fibres. He excised a portion of the sciatic nerve and transplanted it into the subcutaneous tissues of the same animal. Six months later he killed the animal and examined the nerve which had been transplanted. He found among a number of degenerated fibres some which were definitely formed with a medullary sheath showing distinct nodes of Ranvier. He concluded that these fibres had been formed by the tissues of the excised portion of nerve.

He conceives objectors to this deduction maintaining that these nerve-fibres have grown into the sheaths of the transplanted nerve from cutaneous nerves divided in the course of the operation, and meets this objection as follows :—

" Such an explanation is, I think, far-fetched, and assumes an extraordinary affinity between the supposed young nerve-fibres and the old nerve trunk, an affinity which, if established, would, I am sure, secure almost certain spontaneous union of nerve trunks after division, unless very extraordinary barriers were placed between the divided ends.

" The number of cases, however, in which secondary suture has to be performed shows that divided nerves cannot be confidently left to nature to repair, which I am sure might almost uniformly be the case did such strong affinity between young nerve-fibres and the distal degenerated segment exist."

It has, however, been shown that a large nerve trunk on division curls up at the end, forming a contorted leash of fibres. Further, a nerve trunk on division retracts, and a considerable amount of scar tissue is formed. This cicatricial tissue by the time the nerve has begun to regenerate has become dense fibrous tissue. In the case of nerve trunks, therefore, " extraordinary barriers " are the rule. Moreover, in spite of such barriers spontaneous union often occurs, and in animals when the cut is made more cleanly than occurs in an accident in man, and when antiseptics are used, the amount of cicatricial tissue formed is less, and union may be confidently anticipated without suture either immediate or secondary. In the case of small nerve trunks the case is also different; for example, after operations involving extensive skin incisions, anæsthesia is always found on one side of the incision, varying in extent according to the direction of the incision and the number of cutaneous nerves divided. It is, however, the rule for these anæsthetic areas to completely recover.

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* 'Brit. Med. Journ.,' 1904, vol. 2, p. 729.

Again, when a longitudinal incision is made through the rectus abdominis muscle it is very rare to find any permanent paralysis of portions of the muscle, although division of its nerves must be very common. Temporary paralysis, of course, must take place, but there is so little fear of ultimate paralysis and atrophy of the muscle that many surgeons prefer an incision through the rectus to any other method of performing laparotomy.

To further study this point we repeated Kennedy's experiment, with the variation that a portion of a nerve was not only inserted underneath the skin, but a similar portion was inserted into the peritoneal cavity. This was performed as follows: the peritoneal cavity of a cat was opened through an incision in the left hypochondrium, and the stomach drawn up into the wound. The portion of sciatic nerve which had been previously removed from the same animal was laid on the anterior stomach wall. A row of Lembert sutures of catgut were then passed so as to invaginate a groove of tissue around the nerve; the sutures were continued for a short distance at each end of the nerve so as to completely enclose it in a sheath of stomach wall lined by peritoneum. In this way there was no possibility of nerve-fibres growing in from the skin, and but little possibility of their coming from the stomach.

As a control experiment a wisp of catgut was enveloped in the stomach wall in a precisely similar manner.

After 150 days, the animal was killed and the nerves examined. In one case the whole of the nerve tissue had completely disappeared; apparently it had been entirely absorbed by leucocytes, and the suture line was the only sign of the operation.

But in other experiments, where the nutrition of the nerve had probably been more effectually maintained, the nerve could be readily seen both in the stomach wall and under the skin. It was thinner and more transparent than when it was inserted. It was examined in teased specimens and in sections. It can be readily teased out into its constituent fibres, and this method is perhaps the most valuable one of investigating its structure.

When thus prepared, each fibre looks like a long tube; they have no double contour and there is no trace of a medullary sheath. There are very abundant nuclei which take nuclear dyes rapidly and intensely. At the point where a nucleus is situated there is usually no increase in the diameter of the fibre, the nucleus being either central or projecting into a central lumen. In some fibres, on the other hand, there is a distinct swelling at the site of the nucleus, and here there is a considerable amount of protoplasm accumulated around the nucleus. There were no traces of nodes, and nothing resembling an axis cylinder or fibrillæ could be seen. The diameter of the fibres varied from 2·5 to 3 μ. These appearances are illustrated in figs. 1 and 2.

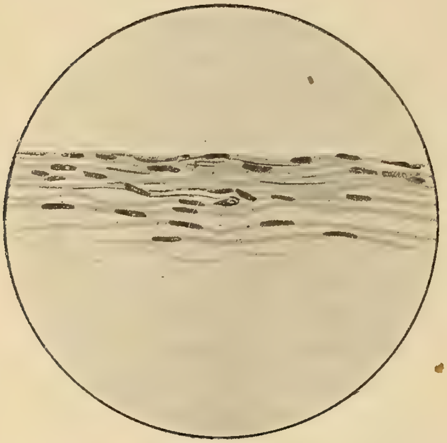

FIG. 1.

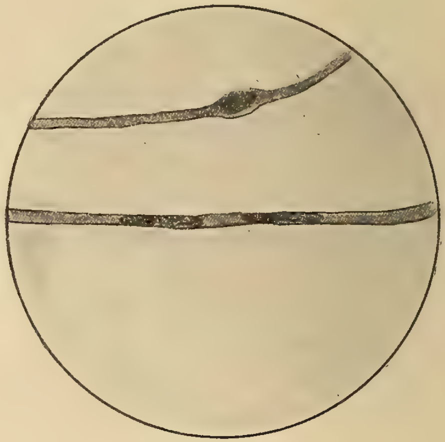

FIG. 2.

The sections (some of which were stained by Cajal's new silver method) showed merely the divided ends of these tubular structures cut across. It is true that the appearance of the long nuclei in transverse section was at first sight suggestive of axis cylinders, but their size, apart from the evidence of longitudinal sections and teased preparations, disproves this view.

The appearances, therefore, are merely those which have been so often described in the peripheral segment of a divided nerve in which regeneration has not occurred, and which all agree is due to neurilemmal activity.

The remains of the nerves which had been transplanted under the skin showed the same appearances. Here also a large number of well formed blood-vessels were found. These grow in not only at the ends, but actually break through the surrounding connective-tissue sheath into the interior. The accompanying drawing illustrates how a blood-vessel breaks through the sheath into the substance of a degenerated nerve bundle.

From this irruption of blood-vessels it is a very small step to the introduction of nerve-fibres into the interior of the transplanted nerve. It seems reasonable to suppose that where the one could penetrate the other would have no difficulty in doing likewise, and this would account for the presence of the medullated nerve-fibres which Kennedy discovered. In our own preparations of transplanted nerves under the skin we could by careful search discover a few medullated fibres, but this was not the case where the nerve trunk had been transplanted under the peritoneum, in which situation invasion by neighbouring nerves is nearly impossible.

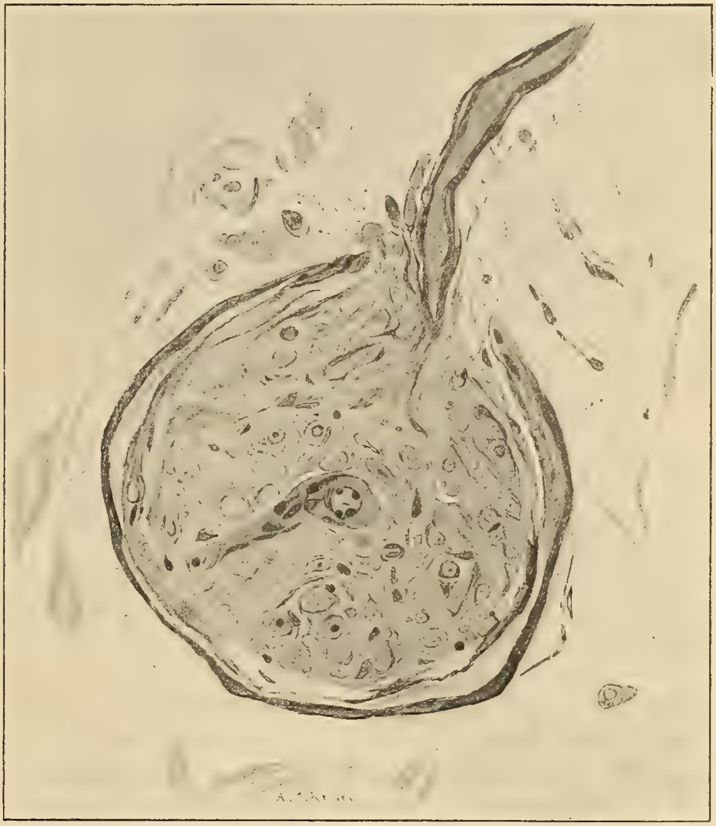

Fig. 3.—A small blood-vessel entering the sheath of a fasciculus of transplanted nerve, as described in the text. 280 diameters.

The connective tissue between and around the bundles of nerve-fibres retains its normal relationship. This is, however, not a property of nerve tissue in particular, for any structure of a similar shape would become enclosed in a sheath of fibrous tissue unless it were completely absorbed. This is well illustrated in fig. 4, which shows a transverse section of a strand of catgut which had been enveloped in the stomach wall.

The connective tissue has organised around the catgut threads, forming a framework which strongly suggests the sheath of nerve fasciculi.

We may sum up our results of these experiments by saying that we have found no evidence of the appearance of new nerve-fibres in nerves which have been transplanted into the peritoneal cavity. Such nerves may either undergo absorption, or be replaced by that variety of tissue which is found replacing nervous tissue proper in the distal segment of a divided nerve.

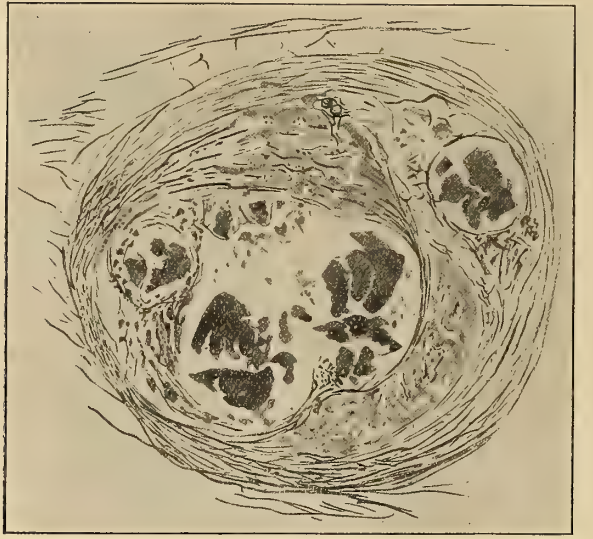

Fig. 4.—Transverse section of strand of catgut which has become surrounded by a sheath of connective tissue, after being transplanted beneath the peritoneal coat of the stomach, as described in the text.

It is at least remarkable that the nerves transplanted by Kennedy into a position where they were entirely dependent on soakage of lymph for nourishment until a blood-supply had reached them should have been so specially fortunate in their powers of regeneration as to produce well-developed medullary sheaths and nodes of Ranvier. It is difficult to understand on the autogenetic theory why so few of the degenerated fibres should regenerate in this way, for all were under precisely the same conditions. Such an occurrence is easy to account for, if our own view is adopted that the well-formed fibres seen had grown into the transplanted nerve from nerve-fibres cut through in the operation.

(3) *Experiments on the Degeneration of Regenerated Fibres.*

These experiments were suggested to us by Professor Gotch, and have been performed once on the monkey and twice on cats. A large nerve (the ulnar in the case of the monkey, the sciatic in the case of the cats) was divided and the ends sutured together with sterilised silk. After a sufficient length of time had elapsed, restoration of function led us to suppose that regeneration had occurred. A second operation was then performed in each case. The animal was anæsthetised, and the nerve was exposed; the union of the two ends was found to have been accomplished, and the nerve was excitable both above and below the junction. A piece of nerve about half an inch long was then excised, an inch or so below the junction. On histological examination of this all traces of degenerated products were found to have disappeared, and it was made up of fine nerve-fibres, many of which had acquired a delicate medullary sheath. After this second operation the wound was closed and the animal allowed to live 10 days longer; it was then killed and the nerve both below and above the second cut was examined. No degeneration was found in the nerve-fibres above the second lesion, but Wallerian degeneration was shown by the Marchi method to have occurred in the medullated fibres of the peripheral portion, which was quite inexcitable. The direction of degeneration is the direction of growth, so this experiment shows that the growth of the nerve-fibres had not started from the periphery centralwards, but in the reverse direction.

In the monkey in which this experiment was performed, the second operation took place 70 days after the first. In the case of the cats a longer interval was allowed to elapse, namely, 147 and 162 days respectively; by this time myelination of the nerve-fibres was more pronounced, and so degeneration was more readily seen by the method employed.

We give below (fig. 5) a drawing of the degeneration seen in a teased preparation of the peripheral segment after the second operation.

We noted an interesting point in these experiments which illustrates the great rate at which the regenerating fibres grow. Although only 10 days had elapsed between the second operation and the killing of the animal, and although quite half an inch of nerve had been excised and no efforts made to approximate the ends, yet there was a well-marked strand of union uniting the two segments, both in the experiment on the monkey, and in one of the two experiments on cats.

We think that these experiments, taken in conjunction with the somewhat similar experiment already alluded to in the work of Langley and Anderson, in which, after union of the two ends of a divided nerve, degeneration in the regenerated fibres set in after a second operation of dividing the central end of the nerve nearer the spinal cord, conclusively prove the regenerated fibres to be in anatomical continuity with the fibres in the central end, and so with the central nervous system. They thus serve to prove the doctrine that the regenerated fibres have grown from those in the central stump.

At the time that we undertook these experiments we were under the impression that they had never been previously performed. But on looking up the literature we find this is not the case. Like so much of the present work set going by the revival of the autogenetic theory, this experiment has also been performed by others. As long ago as 1859 Philippeaux and Vulpian did it, and obtained the same result as we have; we can hardly doubt that this formed one of the factors that later led Vulpian to abandon the autogenetic theory.

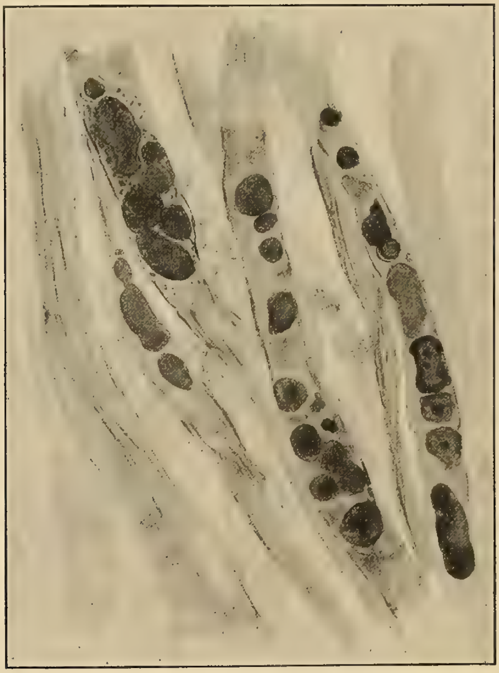

Fig. 5.—Wallerian degeneration of regenerated nerve-fibres. Cat. Drawn from a teased preparation with the camera lucida. Marchi method of staining. Magnified 820 diameters.

Bethe also has repeated the experiment; he states that a nerve which has regenerated autogenetically degenerates afresh when it is again cut; that this degeneration occurs only in the peripheral segment, and that the central end remains intact. According to him this experiment convinces him in a striking way that the integrity of the central end does not depend on its connection with the cells in the central nervous system. This is an excellent example of arguing in a circle, for his first assumption that autogenetic regeneration has occurred is entirely unwarranted.

(4) *Experiments on the Rate of Medullation in Regenerating Nerves.*

Another very important piece of evidence which supports our general views was obtained by examining regenerated nerve-fibres in various parts of their course. In the preliminary announcement of our results we stated:—

"We think in some cases that the more distant the situation from the original point of section, the less perfectly developed the nerve-fibres appear to be; myelination has progressed less in the distal portion of their course."

This conclusion was derived from the examination of a large number of specimens, but as no special measurements of the distance in each case from the point of section had been made, we thought it advisable to examine the matter more systematically.*

We accordingly divided the sciatic nerve of a cat high up in the thigh, and sutured the two ends together. In time the resulting paralysis had largely disappeared, and from this we judged that regeneration of the nerve had occurred, and 84 days after the operation the animal was anæsthetised, and the nerve was found to be excitable both above and below the junction to a weak faradic current, such a current as could just be felt by the tongue. The animal was then killed, and a piece of the sciatic nerve immediately below the junction was placed in Marchi's fluid for subsequent microscopical examination. A piece of the lower end of the posterior tibial at the level of the ankle was similarly treated. The histological study of longitudinal and transverse sections of the two portions of nerve showed a much greater degree of myelination at the upper than at the lower end.

In order to make the experiment still more exact, we proceeded in rather a different way in a second experiment. A possible objection to the first experiment would be that the posterior tibial is a motor nerve, and regeneration of motor fibres is as a rule slower than that of sensory fibres; the greater amount of medullation observed at the upper end of the sciatic might possibly have been due to the fact that there we were chiefly examining sensory fibres. This objection does not really hold, because all the bundles in the upper end of the sciatic were almost equally well myelinated. Still, it appeared wise to meet the objection by somewhat varying the manner of observation. The sciatic nerve of a cat was cut and sutured; 91 days later the animal was killed by chloroform, and during the anæsthesia that preceded death the nerve was found to be irritable to weak faradisation as before, both above and below the junction. In the dissection of the nerves, a large motor and a large sensory bundle in the leg were traced up into the main sciatic trunk, and by dividing the sheath they could be easily separated in the trunk for a considerable distance. A small piece of the upper and lower ends of both the motor and the sensory bundle were then removed, placed in Marchi's fluid, and examined in sections as before.

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* We find that this observation was also made by Langley and Anderson (*loc. cit.*, p. 425). They say, "In one or two of our cases we examined the whole length of the nerve and found that the medullated fibres decreased towards the periphery."

There was a noticeable difference between the amount of medullation in the motor and sensory nerves, that in the latter being, as is usually the case, more advanced. Both, however, showed at their upper ends numerous myelinated fibres; whereas at the lower end, which was 4 or 5 inches distant, myelination was much less distinct. We illustrate these appearances by the figures on the accompanying plate, which show high and low power views of transverse sections of the motor trunk at the two levels.

This second cat, although it was killed a week later than the first, showed less advanced myelination. We have called attention in our previous work to the difficulty of assigning any exact dates, even in the same species of animal, to any particular stage of the regeneration process. The dates vary within somewhat wide limits with the vital reaction of different animals. In the case of the two cats we are at present concerned with, the first animal was a young and lively one, whereas the second animal was large, lethargic, and older. It is therefore not surprising that regeneration should have been more rapid in the first than in the second case.

It can hardly be doubted that the medullary sheath is a developmental appendage of the axis cylinder; it appears in the fibres of the central nervous system where the neurilemma is absent; it degenerates with the axis cylinder when a nerve-fibre is cut, and completeness of function is associated with its appearance in development and with its reappearance in regeneration.

We therefore think we are justified in concluding that the late appearance of the medullary sheath in those portions of the regenerating fibres which are most distant from the place where the nerve was originally cut is a conclusive piece of evidence in favour of the view that the new nerve-fibres have grown from the central end in a peripheral direction.

Von Büngner,* von Notthaft,† Strœbe,‡ and Wieting§ all agree that the formation of nerve-fibres takes place more rapidly near the junction than at the periphery.

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* 'Ziegler's Beiträge,' vol. 10, p. 321 (1891).

† 'Zeitsch. f. Wissen. Zool.,' vol. 55, p. 376 (1892).

‡ 'Centralbl. f. path. Anat.,' vol. 4, p. 49 (1893).

§ 'Ziegler's Beiträge,' vol. 23, p. 63 (1898).

(5) *Experiments on the Influence of Stimulus on Regeneration.*

These experiments were performed on Rhœsus monkeys. A monkey's arm was rendered immobile by the division of a number of the upper posterior roots on one side. In order to be perfectly certain, we usually divided from the second cervical to the second or third thoracic roots inclusive. The anterior cornual cells from which the corresponding motor fibres originate are thus not subjected to stimuli from the periphery and, as Mott and Sherrington were the first to show, the arm is as much paralysed as if the anterior roots had been cut. We have, however, again noticed in some of these animals under the influence of strong emotion (for instance, when the monkey is prevented from reaching with the sound hand a piece of apple) that some efforts were made to move the paralysed limb. These efforts are ineffectual and are limited to associated movements in the upper segment of the limb; fine movements were never noticed. When the animal is living in its cage under ordinary conditions it makes no effort to move the limb, which in successful experiments (*i.e.*, when a sufficient number of roots have been entirely cut through) hangs helpless like a flail.

In the case of one or two of the wilder animals we first operated on, the hanging limb was apt to get injured by their jumping about the cage. Wounds so produced heal with difficulty, and the presence of such wounds necessitated the sacrifice of the animals. Later we obviated such accidents by providing the animals with jackets, the sleeve containing the paralysed limb being well packed with cotton wool.

After the healing of the neck wound produced by the cutting of the posterior nerve roots was accomplished, a matter usually of one or two weeks, a large nerve in the arm (median or ulnar)* was then divided and the ends sutured together. The corresponding nerve was cut and sutured on the non-paralysed side as a control experiment. The animal was finally killed; the interval between the operation and death varied in different experiments, but the best time for making the observation we finally determined to be between 60 and 70 days after the nerves had been cut. This is a date when regeneration is perfectly evident, but not too far advanced; it, therefore, permits of comparative observations on the amount of regeneration.

* We found a considerable anastomosing branch passing from the median to the ulnar nerve in the lower part of the fore-arm; some branches which, still lower down, apparently come off from the ulnar, are really median in origin. A non-recognition of this fact is apt to cause confusion (as it did in some of our early experiments), for after section of the ulnar some of the branches to the last two fingers show no sign of degeneration.

Union of the divided nerves occurs on both sides of the body, and in our early experiments the nerve on the side corresponding to that on which the posterior nerve roots had been divided was found to be the less excitable to the faradic current. Histologically this nerve showed a looser texture, and new nerve-fibres, though present, were somewhat less numerous than on the control side. In these early experiments also we found that the posterior cornual cells in the cervical region were atrophied, and that there was a considerable overgrowth of neuroglia tissue in the posterior horn.

Further examination (by the methylene blue and erythrosin stain) of these spinal cords showed, however, that there had been a considerable number of small hæmorrhages in the cord, sufficient in some cases to cause degeneration in various descending tracts in the cord. It, therefore, became quite possible to explain the effects observed by this complication. We are inclined to think that the hæmorrhages are not due, or not chiefly due, to mechanical injury of the cord during the operation, but are to be explained by the loss of support in the cord tissue which follows degeneration of the entering posterior root fibres.

In several of the later experiments in which cord hæmorrhages did not occur to any great extent, we have been unable to detect any marked changes in the anterior or posterior cornual cells, or any difference to stimulation or in microscopic structure between the regenerated nerves of the two sides.

This conclusion fits in with some experiments of H. K. Anderson;* he showed that in developing animals, section of all the posterior roots connected with a limb exercised no retarding influence on the development of the corresponding anterior roots.

Warrington† has stated that when posterior nerve-roots are cut, the anterior nerve cells undergo the chromatolytic change associated with inactivity. We do not wish to dispute Warrington's observations which apparently were made at an early date after the division of the roots. If the change does occur, it can only be temporary, and in the animals which we have killed at the later dates mentioned, and also some killed at earlier dates (including one killed 17 days after the division of the roots), it was not possible with any certainty to tell by looking at the anterior nerve cells of the two sides which was the side on which the posterior roots had been divided.

In further experiments we sought to cut off the cerebral influence by removing the cortical arm area of the opposite side in addition to dividing posterior roots as before. In this case also the regenerated nerves of the two sides were equally responsive to stimulation, and histological evidence of any marked difference between them was also lacking.

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* 'Journ. of Physiology,' vol. 28, p. 499 (1902).

† 'Journ. of Physiology,' vol. 23, p. 112 (1897—98); vol. 24, p. 464 (1899); vol. 25, p. 462 (1899—1900).

Finally, we still further reduced the action of innervation currents on the anterior cornual neurons by cutting off the stimuli which enter by the posterior roots, as well as those which descend from the brain. The latter was accomplished by combining either a semisection or a complete transection of the cord in the mid-thoracic region with the division of the posterior nerve roots which correspond to the lower limb of one side.

The two sciatic nerves were then divided and sutured and, as before, an interval was allowed to elapse until these nerves regenerated. Here, again, the result was negative. Both nerves regenerated equally well, and both were equally responsive to excitation.

It is, therefore, quite evident that the paths which even under these circumstances remain open (commissural and association tracts) are sufficient to maintain the activity of the anterior cornual cells in the sprouting forth of new axons in a peripheral nerve, although they may be insufficient to induce those cells to send effective impulses along them.

It is also evident that in order to reduce the activity of the anterior nerve cells to a sufficient degree to prevent the regeneration of their axons, it would be necessary to insulate a group of them so as to prevent all impulses reaching them from every part of the nervous system. We did not see our way to accomplish such an operation without interfering with the blood supply of such an island of cord matter. The nearest approach we have obtained to such a condition of things is to be found in those early experiments in which hæmorrhages and neuroglial overgrowth occurred as a complication. Here certainly the diminution in the number of regenerated fibres points to a reduced activity or, it may be, destruction of some of the anterior cornual elements, but in either case these experiments, so far as they go, are in favour of the view that new nerve-fibres grow from central axons, and are not formed autogenetically.

General Conclusions.

We have put forward the foregoing five sets of experiments as a contribution to the discussion now in progress as to whether the regeneration of nerve-fibres is autogenetic or not. These experimental methods approach the subject in different ways, and in no one case was any evidence forthcoming of auto-regeneration.

The facts recorded, taken in conjunction with those published by such observers as Cajal and Langley and Anderson, form on the other hand strong pieces of evidence in favour of the Wallerian doctrine that new nerve-fibres are growths from the central ends of divided nerve trunks. The experimental facts recorded by those who, like Bethe and Kennedy, hold the opposite view are susceptible of easy explanation, mainly on the lines emphasised by Langley and Anderson of accidental and unnoticed connection of the peripheral segments with the central nervous system by means of other nerves cut through in the operation. If such connection is effectually prevented, real regeneration of structure and restoration of function never occurs.

Moreover, the regenerated fibres always degenerate in a peripheral direction, and in a peripheral direction only, when the link that binds them to the central nervous system is again severed. Perhaps the most striking of the facts brought out in our own work is in reference to the development of the medullary sheath ; this appendage of the axis cylinder appears earliest at situations near the point where the ends of a nerve have been joined together, and reaches the distal portions later.

What takes place in the peripheral segment of a divided nerve is a multiplication, elongation and union into long chains of the neurilemmal cells. The same change is even more vigorous at the central termination of the cut nerve; and the view of the phagocytic and nutritive function which we attribute to this sheath has been supported independently by some striking observations of Graham Kerr which we have fully referred to. At the central end this nutritive function is effective and provides for the nourishment of the actively lengthening axis cylinders. At the peripheral end, unless the axons reach it, it is ineffective in so far as any real new formation of nerve-fibres is concerned. If, however, the axons reach the peripheral segment, the work of the neurilemmal cells has not been useless, for they provide the supporting and nutritive elements necessary for its continued and successful growth. The neurilemmal activity appears to be essential, for without it, as in the central nervous system, regeneration does not take place.

According to Graham Kerr the formation of neuro-fibrillæ may possibly take place in the protoplasmic residue of the degenerated axis cylinder; according to Marinesco this property is assigned to the neurilemmal elements themselves, a proposition we regard as extremely improbable, seeing that these elements are mesoblastic. In either case these two observers consider that the neuro-fibrillæ, however formed, are ineffective until they are activated by union with those of the central axons. Our own observations do not entirely exclude this view, but on the other hand (as we have never seen these fibrillæ in the peripheral segment unless union with the central end has occurred) they lend it no support. All our facts are readily explicable, however, on the theory that the nerve-fibres are growths from the central ends of divided nerves.

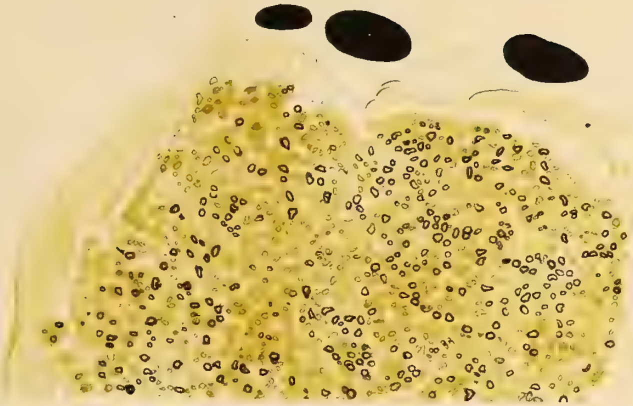

Fig. 6a.

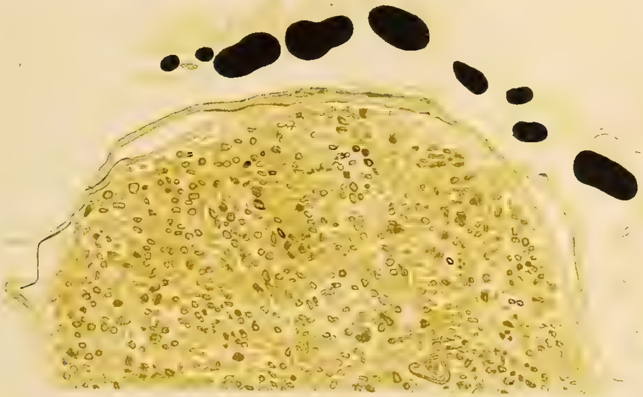

Fig. 7a.

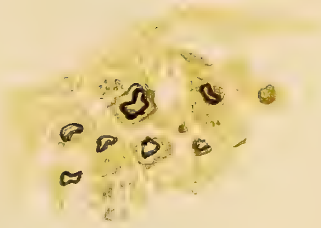

Fig. 6b.

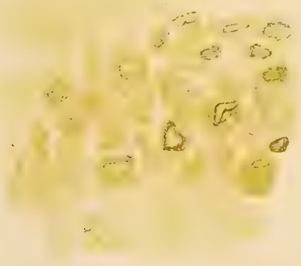

Fig. 7b.

Bale & Danielsson, Lith

Towards the expenses of this research we have received contributions from the Government Grant Committee of the Royal Society, and from the Scientific Grants Committee of the British Medical Association.

DESCRIPTION OF PLATE.

Fig. 6a.—Regenerating motor bundle of cat's sciatic nerve, 91 days after the nerve was divided and sutured. Marchi method of staining. The transverse section shows well-marked myelination in the majority of the fibres; it was taken from the upper end of the nerve. Magnified 350 diameters.

Fig. 6b.—A small portion of the same, magnified 820 diameters.

Fig. 7a.—The same bundle 4 or 5 inches lower down. Myelination much less advanced. Magnified 350 diameters.

Fig. 7b.—A small portion of the same, magnified 820 diameters.

All the drawings were made with the camera lucida.

Observations on the Labyrinth of Certain Animals.

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(Communicated by Professor John G. McKendrick, M.D., F.R.S. Received January 26,—Read February 15, 1906.)

[PLATES 16—18.]

The method of preparing the membranous labyrinth devised by the writer* has made the study of that organ more easy. The difficulty which previously attended the examination of the inner ear was so great that even the extraordinary patience of Retzius only permitted him to complete the investigation of 5 mammals and 11 birds. In more recent times Alexander has examined several more by the microscope, but the total number altogether is still very small.

The writer has already published the results of his investigations in the seal and in man. The results of the examination of 14 other mammals are at present in course of publication and will not be described in this paper, except in so far as they throw light upon the subjects immediately under discussion. It is necessary, however, to refer in general terms to the anatomy of the inner ear in the case of the animals mentioned, in order that that of those described in this paper may be properly understood.

From an examination of 16 mammals the writer has found that the different orders and species present differences in the anatomy of the inner ear of three main types. These are (1) differences in the shape of the cochlea; (2) differences in the size of the perilymphatic space of the semicircular canals; (3) differences in size of the otoliths.

The cochlea appears in the mammals under two types in addition to the peculiar type of the organ as found in the monotremes. These types are the sharp-pointed cochlea which is found in the carnivora and the rodents, and the flattened cochlea which is found in man, the monkeys, the lemurs, the ungulates and the cetacea. In the case of the carnivora one exception was found to this rule, the exception being the seal. This animal possesses a cochlea which is rather bowl-shaped than pointed like those of the cat, dog, and puma. There were no exceptions in the case of the ungulates, the least flattened cochlea being possessed by the pig, but the difference between it and the same organ in the other ungulates was found to be slight. There were no exceptions to the rule in the rodents: the rat, the mouse, the guinea-pig and the rabbit all being possessed of sharp-pointed cochleæ. Three monkeys were examined and no exception was found, the cochlea being in every case of the flattened type and very like that of man, but smaller. The organ in the lemur was also of the flattened type, as was that of the porpoise.

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* 'Journal of Anat. and Physiology,' vol. 37, p. 379.

The perilymphatic space of the semicircular canals proved to be a very interesting study. Our ideas of the anatomy of the labyrinth have depended so much upon the investigation of the organ in the human subject that it is not surprising that errors have crept in by assuming that certain features found in him will also be found in the lower animals. The present is a case in point. The perilymphatic space in the canals of the human subject is large and well developed, and it has been assumed that this would be true of other animals. But such is not the case. It so happens that man is one of the exceptions to a general rule. In mammals the rule is that the perilymphatic space is either very small or even completely absent in the canals. The exceptions to this rule are:—man, the monkeys, and the seal. No doubt there are other exceptions; indeed, another falls to be recorded in this paper, but a sufficient number of examples have been examined to assert that the rule above given is a fairly general one.

In spite of the existence of this general rule, however, it is probable that the original type of the mammalian labyrinth possessed a well-developed perilymphatic space in the canals. The chief reason for this belief is, that in the reptiles and birds this is the type invariably found in all the animals of those divisions which have been examined. A more definite statement will be possible when the labyrinth of the monotremes has been investigated in regard to this feature. One of these is at present in course of preparation, and the results of the examination will be published later. At present we may regard the presence of a well-developed perilymphatic space in the canals as indicating a labyrinth of a more ancient type. This, of course, does not mean that the particular species which possesses such a labyrinth is therefore an ancient species. Obviously, man is a very recent species of animal, although he possesses the space referred to above. It merely means that man's progenitors have not dispensed with the space when other species of mammals were in process of losing it.

The presence or absence of this space is therefore a valuable guide to the relationship of different orders and species of mammals. Its value is enhanced by the fact that the space does not appear to have any physiological function. It is found in animals which have great delicacy of movement, and in animals which have not, as is evidenced by the monkeys on the one hand and by the sloth on the other. It is found in animals which migrate, such as the seal, and is absent in others which also migrate, such as the porpoise. In so far as the function of equilibration is concerned, the space appears to be of no value, for it is found in the climbing monkey, and is absent in the lemurs, which also are nearly all arboreal. In short, the space may apparently be retained or dispensed with according to the necessities of the case. What these necessities are we do not yet know, but it is very probable that if more room is required for the surrounding structures the perilymphatic space of the canals will disappear.

The otoliths of mammals have hardly in previous times been examined carefully, it being assumed that they are small, a natural assumption from the fact that in the human subject they are of very trivial dimensions. This assumption is in the main correct, but the writer has already discovered two exceptions, the porpoise and the seal, and another falls to be recorded in this paper, the kangaroo.

The labyrinths which are described in this paper are: the lion, the Indian gazelle, the three-toed sloth, and the kangaroo among mammals; the crested screamer and the ostrich among the birds.

The Labyrinth of the Lion (Felis leo). (Plate 16, fig. 1.)

The membranous labyrinth of the lion differs in hardly any respect from that of other felidæ except in the matter of size. The cochlea is of the sharp-pointed type, and measures 9 mm. in diameter in the lowest whorl. The second whorl measures 6 mm. in diameter. The scala tympani shows a marked bulging at its lower extremity just before it reaches the round window. This is a common feature in the carnivora and in some of the other mammals. The slant height of the cochlea from the upper margin of the round window to the apex of the organ is 4·5 mm. in length. There are three turns in the spiral of the cochlea, this being a fraction of a turn more than that of the puma, and a quarter of a turn less than that of the dog. The cat, like the lion, has three turns in its cochlea.

The vestibule measures 5 mm. in its longest diameter, and there are no otoliths in the cavity of a size sufficient to be recognised by the naked eye. The oval window is elliptical in shape, being in this respect like those of the other carnivora, except the seal, in which the aperture is semicircular (if the contradiction in terms may be excused).

The semicircular canals are very regular in shape, rounded, and without any noticeable irregularities such as are found in some mammals. Each canal lies in one plane, there being no lateral deviations. The superior is the largest of the canals. It measures 6·5 mm. from limb to limb internally, and 7·5 mm. externally. The height of the vertex of the canal from the vestibule is 6 mm., and the diameter of the canal itself at the vertex is 0·75 mm.

The posterior canal lies in a plane almost at right-angles to that of the superior canal. It is somewhat smaller than the latter. Its diameter, measured internally, is 5·5 mm. in length, and measured externally is rather less than 7·5 mm. The height of the vertex of the canal above the vestibule is 5·5 mm. and the diameter of the canal itself at the vertex is 0·5 mm.

Unfortunately the horizontal canal was broken in the process of preparation, but it was noticed that it was distinctly smaller than either of the other two. The plane of this canal is relatively low and, indeed, at its posterior extremity it appears to open into the ampulla of the posterior canal. This low level of the plane of the horizontal canal is very common among mammals, and in none of the mammals examined by the writer is the plane of the canal so high relative to the posterior canal as in the monkeys and in man. In the two latter the space enclosed by the curve of the posterior canal is almost bisected by the plane of the horizontal canal.

The perilymphatic space is almost entirely absent from the canals of the lion, indeed it can only be seen in the angles formed by the ampullæ of the canals with the canals themselves. This feature is common to the carnivora that have been examined with the single exception of the seal. In the latter the space is well marked.

The length of the whole labyrinth of the lion is 17 mm. The cochlear portion is large relative to that portion formed by the vestibule and canals. In this respect the lion resembles the cat and other carnivora. A condition exactly the reverse of this is found in the lemur.

The angles which the planes of the canals form with one another are rather large in the lion. That is to say, the canals diverge widely from one another, much more so than in man. This divergence of the canals is greater in the lemur than in any animal which I have yet examined.

The Labyrinth of the Indian Gazelle (Gazella Bennetti). (Plate 16, fig. 2.)

The membranous labyrinth of the Indian gazelle resembles that of the other ungulates in its general outline. There are, however, some unexpected differences.

The organ measures 14 mm. in extreme length from the outermost point on the vertex of the posterior canal to the innermost point on the lowest whorl of the cochlea. The diameter of the lowest whorl of the cochlea is

7 mm., while that of the second whorl is 3·5 mm. The diameter of the tube of the cochlea immediately in front of the round window is also 3·5 mm., the scala tympani showing a marked bulging downwards similar to that found in other ungulates and in most of the mammals. The slant height of the cochlea measured from the upper margin of the round window to the apex of the organ is 5·75 mm. The aqueduct of the cochlea is quite unlike that of any of the mammals which I have hitherto examined. Instead of being comparatively straight, as in its near ally the antelope, it is sharply curved. A large vein accompanies the aqueduct out of the cochlea and, to judge from the photograph, the blood is carried away from the cochlea by this vein. This disposition of the veins of the organ is different from that of the arteries, which are supplied by the internal auditory artery which enters the labyrinth by way of the internal auditory meatus.

The shape of the cochlea differs in no way from the general type of the ungulates, that is to say, it is of the flattened type. It consists of two and a-half turns.

The vestibule of the Indian gazelle is also like that of the other ungulates. It measures 3·75 mm. in its longest diameter and does not contain any otoliths of a size sufficient to be recognised by the naked eye. The oval window measures 2·75 mm. in its longest axis.

The semicircular canals of the Indian gazelle differ from those of the other ungulates in one important respect. The perilymphatic space is much more marked than in either the antelope, the sheep, or the pig. In the last-mentioned this space does exist throughout the whole length of the canals, but is so small that at parts it can hardly be seen; in the sheep and antelope it cannot be seen at all in a large portion of the length of the canals. In the gazelle, however, the space can be traced easily round the whole course of all the canals. It is not so large as in man, the monkeys, the seal or the sloth. In their general appearance the canals show much the same features as those of the other ungulates, apart from the fact of the large perilymphatic space. They are regularly curved in outline, the pig being the one exception to this rule in the ungulates.

The diameter of the superior canal, measured internally, is 4·75 mm., while externally the diameter is 6 mm. The height of the vertex of the canal above the vestibule is 6 mm. and the diameter of the canal itself at the vertex is 1 mm. The posterior canal measures 4 mm. in its internal, and rather more than 6 mm. in its external diameter. The height of the vertex of the posterior canal above the vestibule is 5 mm. and the diameter of the canal itself at the vertex is 1 mm. The external canal measures 3·5 mm. in its internal and 5 mm. in its external diameter. The height of the vertex of the canal above the vestibule is 3·5 mm. and the diameter of the canal itself at the vertex is 1·25 mm.

The canals of the Indian gazelle do not diverge from one another at quite such large angles as those of the antelope and the sheep. In this respect they resemble the canals of the pig, though the divergence is still less in the last-mentioned animal.

The Labyrinth of the Three-toed Sloth (Bradypus tridactylus).

(Plate 17, fig. 3.)

Hitherto the writer has only had the fortune to obtain one example of the edentata, that being the three-toed sloth. To judge from this example, the labyrinth of these peculiar animals will be interesting and instructive to study.

The cochlea is intermediate in shape between the flattened and the sharp-pointed type, but inclining rather to the former. In this respect the organ differs from the great majority of mammals, since, as has been already pointed out, there is only one other animal among all those which have been examined which does not fall clearly into one of the two types, that animal being the seal.

The labyrinth measures 10 mm. in extreme length from the outermost point on the posterior canal to the innermost point on the lowest whorl of the cochlea.

The lowest whorl of the cochlea is 5·5 mm. and the second 3·75 mm. in diameter. The diameter of the tube of the cochlea in front of the round window is 2 mm. and there is no marked bulging of the floor of the scala tympani in this region. In the latter respect the labyrinth resembles that of man and the monkeys; but the aqueduct of the cochlea of the sloth is much thicker than in these two orders. There are two and a-half turns in the cochlea and the slant height of the organ, measured from the upper margin of the round window to the apex, is 3·75 mm.

The longest axis of the oval window is rather less than 1·5 mm. in length, and the longest diameter of the vestibule is 3 mm. There are no otoliths of a size sufficient to be recognised by the naked eye.

The canals of the sloth are quite unlike those of any mammal which the writer has had the opportunity of examining. They are not semicircular in shape, the horizontal canal being the only one that approaches this form, and even it is irregular. The posterior and the superior canals are quadrilateral, or roughly so. The common limb of these two canals arises from each respectively at right angles, because there is none of that curving downwards to meet each other as they approach, such as is found in all other mammals which have been examined. Similarly, the canals as they approach their ampullary extremities do not curve towards the vestibule, but turn suddenly downwards at right angles a short distance before they dilate into the ampullæ.

The sloth, in common with man, the monkeys, and the seal, has a well-developed perilymphatic space in all the semicircular canals. According to the view expressed by the writer this indicates a relatively ancient type of labyrinth.

The internal diameter of the superior canal measures 3 mm., and the external diameter 4·5 mm. The height of the vertex of the canal above the vestibule is 2·25 mm., and the diameter of the canal itself at the vertex is 1 mm. The internal diameter of the posterior canal is 2·5 mm., and the external diameter is 4·5 mm. The height of the vertex of the canal from the vestibule is 2·5 mm., and the diameter of the canal itself at the vertex is 1 mm. The external canal is much the smallest of the three, measuring only 1·5 mm. in diameter internally and 3·5 mm. externally. The height of the vertex of this canal above the vestibule is only 1·5 mm., and the diameter of the canal itself at the vertex is 1 mm.

The angles at which the canals diverge from one another are smaller than in any of the mammals which have been examined and this gives the canals the appearance of having been pressed somewhat together. In addition to this it will be seen, on comparing the measurements or on examining the photograph, that the vestibule and canals occupy a smaller proportion of the whole labyrinth than in most mammals. It is exactly the reverse, for example, of the condition found in the lemur, where the canals are very long and slender. It may be that this small size of the canals, associated with their irregular shape, may be in some way related to the sloth's clumsy and slow movements. The life which they lead, with the body inverted, as it almost continually is, may also be connected in some way with the curious development of these organs.

The Labyrinth of the Brush-tailed Wallaby (Petrogale penicillata).

(Plate 17, fig. 4.)

The labyrinth of the marsupials is not so divergent in structure from that of the general type of mammalian labyrinth as might be expected in an order of animals so far removed from most of the present divisions. It is, for example, less peculiar than that of the sloth and far less peculiar than that of the monotremata, the cetacea, or the seal.

The whole labyrinth measures 8 mm. in length from the outermost point on the vertex of the posterior canal to the innermost point on the lowest whorl of the cochlea.

The diameter of the lowest whorl of the cochlea is rather less than 4·5 mm. in length, while that of the second whorl is only 2·5 mm. in length. There is a marked bulging of the floor of the scala tympani in the region of the round window and the tube of the cochlea at this point measures 2·5 mm. in diameter. There are a little more than two and a-half turns in the cochlea and the slant height of the organ from the apex to the upper margin of the round window is 3·75 mm. The general shape of the cochlea is like that of the ungulates and more particularly like that of the pig. The aqueduct of the cochlea is a short tube of about 1·5 mm. in length, this being perhaps the most peculiar feature of the organ in this animal. The aqueduct is thick in proportion to its length and is straight. It is not triangular as in most of the mammalia, but is flattened from above downwards.

In proportion to the rest of the labyrinth the vestibule is rather large, measuring 4·0 mm. in its longest diameter. The longest axis of the oval window is 2·0 mm. in length. There are two otoliths of considerable size in the vestibule. They are larger than those found in any other mammal, with the single exception of the seal, even the porpoise not being an exception in this respect. Both otoliths lie on the inner wall of the cavity, one anteriorly immediately behind the ampullæ of the superior and external canals, while the second lies a little below the first. They are both flat, and are of irregular outline.

The semicircular canals are very like those of other mammals and are beautifully regular in outline, reminding one of the same structures in the antelope, though of course much smaller. The superior canal measures 3·5 mm. in its internal, and 5 mm. in its external diameter. The height of the vertex of the canal above the vestibule is 3 mm., and the diameter of the canal itself at the vertex is 0·5 mm. The internal diameter of the posterior canal is 3 mm., and the external diameter of the same canal is 4·5 mm. The height of the vertex of the canal above the vestibule is 3 mm. and the diameter of the canal itself at the vertex is 0·5 mm. Thus the posterior canal is smaller than the superior. The smallest of the three canals is the external, which measures 3 mm. in its internal and 4 mm. in its external diameter. The height of the vertex of the canal above the vestibule is 2·75 mm., and the diameter of the canal itself at the vertex is 0·5 mm.

There is a very small perilymphatic space in the canals; it is more noticeable in the angles formed by the ampullæ with the canals themselves. In this respect therefore, the labyrinth is like that of the ungulates, with the exception of the gazelle.

The labyrinth of the kangaroo is not of such an ancient type as we might have expected, save in one respect—the presence of large otoliths. It is unfortunate that the writer has only been able to obtain one example of the marsupials, and it may be that in other species of this order a more ancient type of labyrinth may yet be found. In this connection it should be pointed out that the diprotodont class of marsupials is generally considered to be of more recent origin, and this, if true, may account for the fact that the labyrinth of the kangaroo is not of such an ancient type as we might have expected.

The Labyrinth of Birds.

It is quite outside the scope of this paper to describe in detail the typical labyrinth of birds. That work has already been done by several writers and n particular by Retzius, to whose writings the reader is referred. The present purpose is to show the likenesses and differences which exist between the various species. The reader need only be reminded that there are, as in almost all vertebrates, three canals which occupy nearly the same position relative to one another as they do in the mammals. On the whole, perhaps, they vary more in their disposition than do those of the mammals. One feature seems to be peculiar to the labyrinth of the bird: the horizontal canal, instead of terminating almost in the plane of the posterior canal, passes underneath the latter and projects backwards behind the plane in which it lies. The result of this arrangement is that the two canals form a cross of which the limbs are almost at right angles to one another. At the point of crossing there is a channel of communication between the two canals and it has been supposed that this is a constant feature of the labyrinth of the bird. It certainly is a very general condition, but, as will be seen later, it is not absolutely true of all the birds.

The perilymphatic space of the semicircular canals of the bird is always well marked so far as the present writer's investigations go. This is a notable distinction of the labyrinth of the bird from that of the mammals.

The shape of the canals in the bird is in general that of an ellipse rather than of a semicircle. The superior canal, however, varies considerably in shape, and no constant types can at present be described. This canal is always the largest, or rather has been found to be so, in all the examples hitherto examined.

The vestibule is relatively small. It usually contains otoliths of a size easily seen by the naked eye. They are flattened and are of various shapes. The most usual number of otoliths found in the vestibule is two.

The cochlea of the bird consists of a more or less straight tube. It passes a little downwards and then inwards and forwards. There is usually a slight curve on it, with the concavity directed backwards and a little upwards. The main branch of the cochlear nerve runs along the posterior border of the cochlea and sends filaments forwards to the organ of Corti. At the tip of the cochlea, however, the nerve radiates out like a fan into the lagena, and at this spot there is in many birds a saddle-shaped otolith with the concavity directed outwards.

The ampullæ of the semicircular canals of birds differ from those of the mammalia. They are usually set at a more acute angle with the canal as it leaves them. The nerve to the ampulla cuts into it, so to speak, and partially divides the ampulla into two portions, one adjacent to the vestibule and the other adjacent to the canal itself.

The Labyrinth of the Crested Screamer (Cariama cristata). (Plate 18, fig. 5.)

The term " crested screamer " is applied to two quite different birds. That from which the labyrinth was taken and prepared by the writer and forms the subject of this description, is closely allied to the cranes and has no relation to *Chauna cristata*, which is related to the ducks and geese. According to some ornithologists, *Cariama cristata* is more closely allied to the hawks than to the cranes. The bird lives in the southern parts of Brazil and Paraguay. It will only fly if hard pressed, the usual method of progress being a stooping run. In some of its habits it is like a bustard, its note is a scream or bark. It lives in the high grass and the habits of the bird are diurnal.

The labyrinth is rather large for the size of the bird, measuring 15 mm. from the uppermost point on the vertex of the superior canal to the innermost point at the tip of the cochlea.

The cochlea is very straight in this bird, the usual curve being almost entirely absent. It measures 6 mm. in length from the front of the round window to the tip of the organ. This is a long cochlea for a bird, that of the ostrich being the only one out of the nine which have been examined that is longer. The diameter of the tube of the cochlea immediately in front of the round window is 2 mm. in length. At the tip of the cochlea the nerve widens out into a spade-shaped structure, and a minute straight otolith is present at this point.

The vestibule measures 3 mm. in its longest diameter, and there is a very small otolith present in the utricle. The macula neglecta is to be seen close to the opening of the cochlea.

The superior canal is much the largest and, roughly speaking, is in the form of an ellipse, with the long axis directed upwards and backwards.

The superior canal measures 6 mm. internally and 8 mm. externally. The height of the vertex of the canal above the vestibule is 6 mm. and the diameter of the canal itself at the vertex is 1 mm. The posterior canal is next in size to the superior and is also in the form of an ellipse. It measures 4 mm. in its internal diameter and 6·5 mm. externally. The height of the canal above the vestibule is 3 mm., and the diameter of the canal itself at the vertex is 1·5 mm. The common limb of the superior and posterior canals is very much shorter than is the case in any of the mammals. The horizontal canal is the smallest of the three and is elliptical in shape, the major axis being in the antero-posterior plane. It measures 3·5 mm., in internal and 6 mm. in external diameter. The height of the canal at the vertex from the vestibule is 3·25 mm. and the diameter of the canal itself at the vertex is 1·5 mm.

As is the case in most of the birds there is a communication between the horizontal and superior canals at the point at which they cross, but the opening is very small. The horizontal canal does not project so far backwards in the crested screamer as in the majority of birds and this gives to these two canals an arrangement similar to that found in the mammals.

The perilymphatic space is well marked in all the canals.

The Labyrinth of the Masai Ostrich (Struthio masai).

(Plate 18, fig. 6.)

In so far as the writer's investigations go, no examination has been made of the labyrinth of this division of the order of birds. It is, however, one of the most interesting on account of the fact that these birds are less distantly removed from the reptiles than any others.

Unfortunately both the labyrinths which the writer obtained were broken, and it is therefore impossible to give a description of the complete organ. The most important parts were not destroyed.

So far as can be judged from the broken specimens, the whole organ measures about 17 mm. in length. The cochlea is relatively small and only measures 6·5 mm. in length. It has a stumpy appearance and the backward curve is very well marked. The nerve spreads out fanlike at the tip of the organ and at this point there are several small otoliths arranged in the form of a saddle with the concavity directed outwards. This plurality of the otoliths at the apex of the cochlea is not found in any of the other birds which have been examined, though the single otolith which is so often found here is also saddle-shaped and has the concavity in the same direction. The diameter of the tube of the cochlea just in front of the round window is rather more than 2 mm.

The vestibule is an irregular cavity and measures a little more than 3 mm. in its greatest diameter. It contains two large otoliths. Both of these are flat. The largest, which lies close to the ampullary openings of the superior and horizontal canals, is roughly circular in shape. The second and smaller one is almost square and lies about 1 mm. below and internal to the first. Both the otoliths are milk-white in colour. The oval window measures 2·5 mm. in its longest diameter.

As far as can be judged from the broken specimen, the superior canal is in the form of an ellipse, with the longest diameter lying backwards and upwards. It is much the largest of the three, measuring 7·5 mm. in its internal and 11 mm. in its external diameter. The height of the vertex of the canal above the vestibule is about 9·5 mm. and the diameter of the canal itself at the vertex is 1·5 mm. The posterior canal is also in the shape of an ellipse, with the long diameter in a vertical plane. It measures 5·5 mm. in its internal and 8·5 mm. in its external diameter. The height of the vertex of the canal above the vestibule is only 3·5 mm. and this gives to the canal a somewhat squat appearance. The diameter of the canal itself at the vertex is 1·5 mm. The horizontal canal is the smallest of the three and, like the posterior, is of a squat appearance. It measures rather more than 4 mm. in its internal and 8·5 mm. in external diameter. The diameter of the canal itself at the vertex is 1·25 mm.

A very interesting feature of the canals of the ostrich is the fact that there is no communication between the posterior and horizontal canals at the point at which the arch of the latter passes under that of the former. There is a distinct though narrow interval between them which is filled up with bone in the unprepared subject. This feature of the labyrinth of the ostrich is unique in birds so far as present investigations have shown, but it may be found in other birds of the same class. A specimen of the rhea is now in course of preparation and it will be interesting to see if this feature is repeated in that bird.

EXPLANATION OF PLATES 16—18.

Each labyrinth is represented by two plates. The upper plate is a half-tone reproduction from a photograph of the organ. The finer details are shown in this plate, but the three dimensions which the organ occupies are not of course appreciable and the comparative sizes are not represented, as the degree of magnification varies in each. The rough outline drawings below give, to a certain extent, the sense of three dimensions. The comparative sizes of the objects are also shown in these drawings, each being magnified about four times. The lettering and magnification refer only to the outline drawings. The position of the object is not always the same in the drawing as in the photograph.

The lettering is the same for all the plates.

n. c.	Cochlear nerve.
a. h.	Ampulla of the Horizontal Canal.
a. s.	Ampulla of the Superior Canal.
e. s.	Endolymph space of Superior Canal.
p. s.	Perilymph space of Superior Canal.
v.	Vestibule.
a. p.	Ampulla of the Posterior Canal.
o.	Otolith.
p.	Posterior Canal.
p. h.	Perilymph space of the Horizontal Canal.
e. h.	Endolymph space of the Horizontal Canal.
c.	Cochlea.
x.	Fenestra rotunda.
h.	Horizontal Canal.
p. p.	Perilymph space of Posterior Canal.
e. p.	Endolymph space of Posterior Canal.
a. c.	Aqueduct of the cochlea.

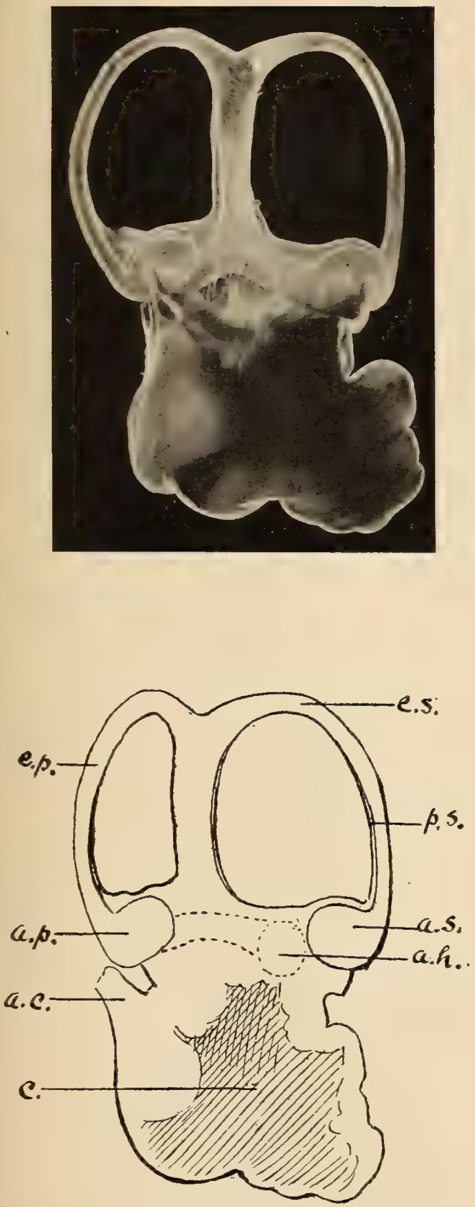
e.s.
e.p.
p.s.
a.p.
a.s.
a.h.
a.c.
c.

FIG. 1.—Membranous Labyrinth of the Lion, *Felis leo.* × 4.

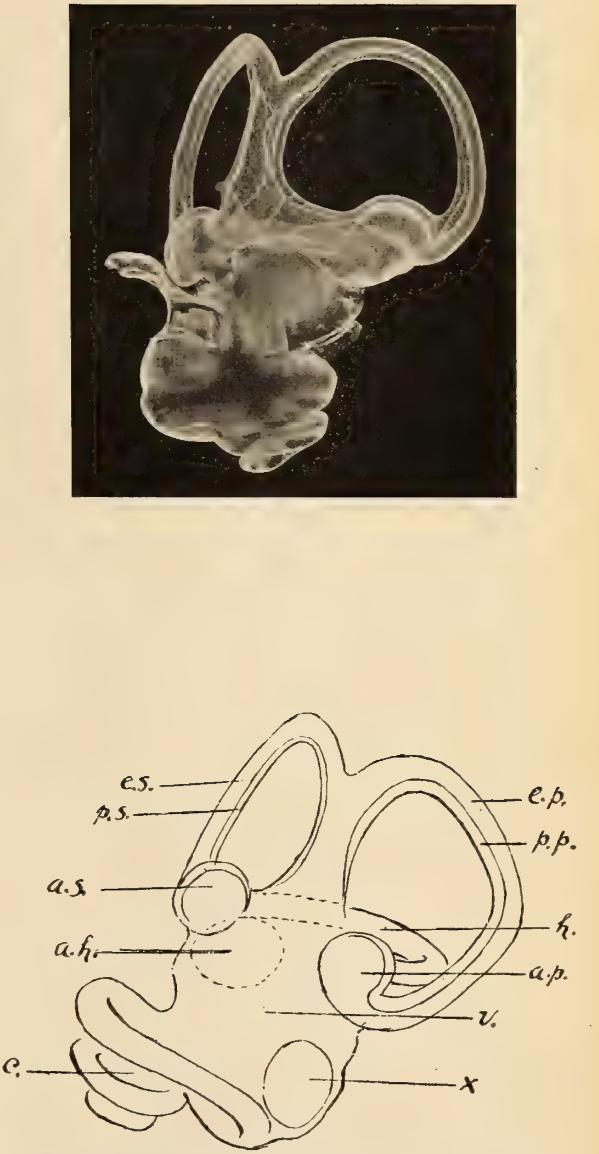
e.s.
p.s.
e.p.
p.p.
a.s.
h.
a.h.
a.p.
v.
c.
x

FIG. 2.—Membranous Labyrinth of the Indian Gazelle, *Gazella Bennetti.* × 4.

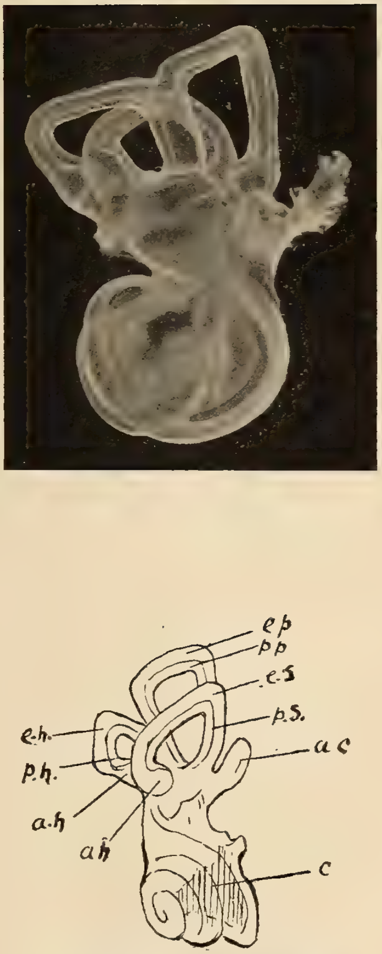
ep
pp
e.s
p.S.
a c
e.h.
p.h.
a.h
ah
c

Fig. 3.—Membranous Labyrinth of the Three-toed Sloth, *Bradypus tridactylus*. ×4.

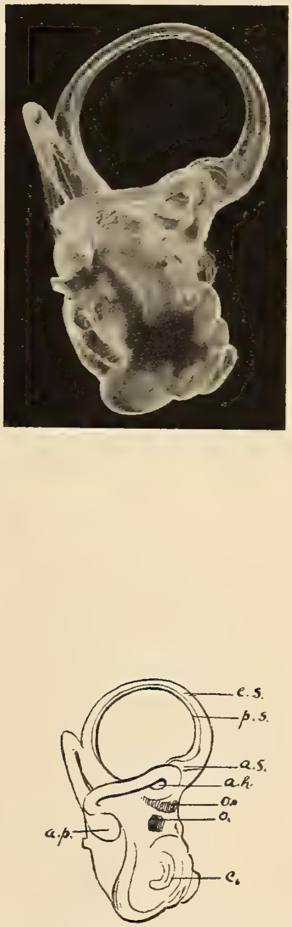
e.s.
p.s.
a.s.
a.h.
o.
o.
a.p.
c.

Fig. 4.—Membranous Labyrinth of the Brush-tailed Wallaby, *Petrogale penicillata*. ×4.

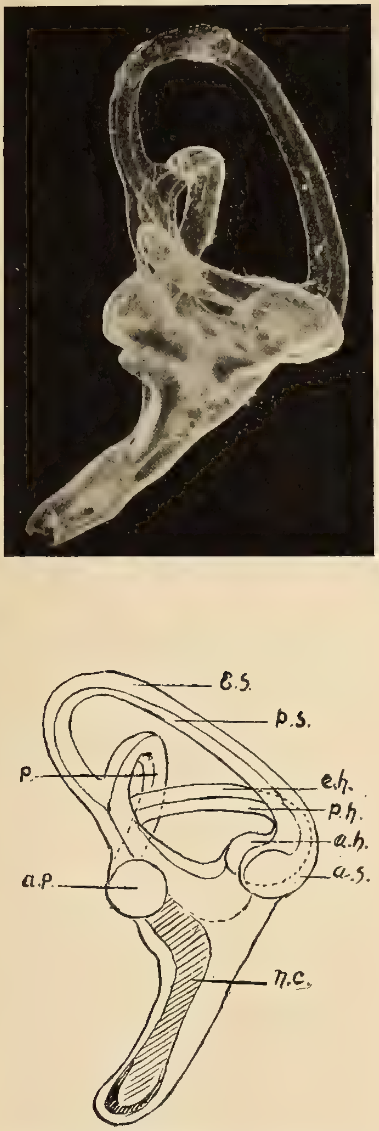
e.s.
p.s.
p.
e.h.
p.h.
a.h.
a.s.
a.p.
n.c.

Fig. 5.—Membranous Labyrinth of the Crested Screamer, *Cariama cristata.* ×4.

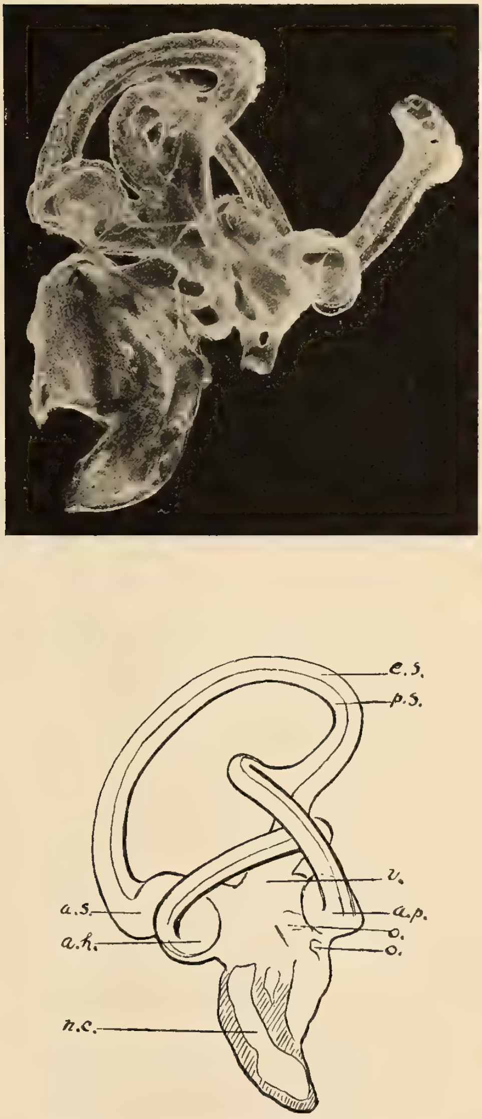
e.s.
p.s.
v.
a.s.
a.p.
a.h.
o.
o.
n.c.

Fig. 6.—Membranous Labyrinth of the Masai Ostrich, *Struthio masai.* ×4.

On the Main Source of "Precipitable" Substance and on the Rôle *of the Homologous Proteid in Precipitin Reactions.*

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(From the Physiological and Pathological Laboratories of the University of Sydney.)

In suitable conditions outside the animal body the most obvious result of the interaction of a precipitin antiserum with its homologous proteid is the appearance of a flocculent precipitate in previously clear solutions. The accepted explanation is that the antiserum contains a precipitating substance or precipitin, through the agency of which the precipitable substance, represented by the homologous proteid, is thrown out of solution. It would generally be admitted that the precipitin to some extent enters into the constitution of the precipitate, since it is through combination with definite quantities of precipitin that the proteid is by most observers supposed to be rendered insoluble. Nevertheless, even if that view of the interaction be adopted, and still more if the precipitin be regarded as the analogue of a ferment or as a non-proteid body altogether, it has been generally taken for granted that the precipitate is constituted essentially by the precipitation of the test proteid, and this conception of the source of the "precipitable" substance has dominated all hypotheses and statements regarding the nature of precipitin reactions.

So far, however, as our observations go, we are led more and more definitely to the conclusion that the great bulk of the substance that is thrown out of solution and that goes to form the precipitate, is supplied by the antiserum and not by the test proteid, and that, whatever part in the interaction is played by the test proteid, it is not merely that of supplying a substance to be thrown out of solution by the precipitin antiserum.

That the antiserum itself is the main source of the "precipitable" substance is indicated by the fact that quantities of the test proteid many times too minute to yield an appreciable precipitum with ordinary proteid precipitants may still yield distinct precipitates on the addition of a suitable antiserum. Even if the whole of the test proteid were thrown out of solution, it would be altogether insufficient to account for the mass of the precipitum obtainable in the latter instances. But it can be shown that the test proteid is not all removed from the solution even when minute quantities interact with antiserum to yield definite precipitates. On the contrary, when the clear superfluid is transferred to a fresh tube and a second dose of antiserum is added, there is deposited a second precipitate, and this process has been repeated six times, each fresh addition of antiserum resulting in a fresh precipitate. In these circumstances it is hard to conceive an adequate source of "precipitable" substance other than the antiserum. Supported by other facts also, this conception of a precipitable content in precipitin antisera is not only in harmony with the known phenomena of precipitin reactions, but appears to offer a more consistent explanation of these phenomena and of certain anomalies that arise on the assumption that the precipitable substance is derived wholly or mainly from the test proteid.

Our experiments are based on the exact measurement of the substances concerned. The interacting quantities were often very minute, and we have been aided in their measurement by the use of dried material. When it was more convenient to employ undried material, a known volume was dried and weighed, so that our results might be expressed in terms of the weights of dried equivalents. We have independently determined the fact, recognised by the majority of observers, that careful desiccation of the substances concerned does not impair their efficiency.

At a room temperature averaging 18° C. and in the dilutions employed by us, we have repeatedly noted that a time interval of less than 48 hours may be insufficient for complete interaction, though in certain circumstances the reaction may be regarded as complete in 24 hours.

For such prolonged exposures, and more especially when the same solutions were tested again and again by repeated addition of proteid or of antiserum, it became imperative to exclude bacteria as effectively as possible. This we were able to do by collecting and storing our material aseptically, by sterilising all apparatus and saline solutions employed, by filtration through porcelain of suspected solutions, and generally by taking the usual precautions against bacterial contamination such as plugging the tubes with sterile cotton wool. The only stage at which contamination was likely to occur was that of shaking the tubes to ensure thorough mixing of the solutions. In the relatively few instances in which bacterial growths appeared we had no difficulty in distinguishing the sediment due to bacteria from the flocculent matter thrown out as a result of precipitin interactions. Nevertheless, when small precipitates were in question, we rejected contaminated tubes.

In reading the precipitates we have made a careful approximation to the length of the tube occupied by the deposit, a graduated scale being held alongside. In any one experiment it is possible to compare and classify the precipitates with accuracy, and in different experiments or in different stages of the same experiment a reliable comparison between the deposits may be made by taking sufficient intervals between the readings such as might be represented by traces, 0·5 mm., 1 mm., 2 mm., and other multiples of 1 mm.

Ordinary test-tubes and samples of the small test-tubes commonly employed in precipitin reactions we found to be unsuited for the reading of the smaller precipitates. Consequently, we were obliged to make our own tubes from narrow glass tubing not exceeding 4 mm. in diameter, the melted ends being simply allowed to fall together in the flame. For special purposes tubing of about 5 mm. diameter was employed.

The following experiments afford a comparison between the precipitates obtainable from the interaction with precipitin antisera of varying quantities of proteid in solution and those obtainable from corresponding proteid solutions by addition of proteid precipitants.

In *Experiment I* two corresponding series of tubes were prepared containing from 0·05 to 0·00000005 gramme dried horse serum in 0·5 c.c. saline solution (0·75 per cent. sodium chloride), so that the dilution of dried horse serum in 0·5 c.c. saline varied from 1 in 10 to 1 in 10,000,000. A control tube containing 0·5 c.c. saline alone was also prepared. To each tube of the first series and to the control was added 0·01 gramme dried antiserum (prepared in a rabbit by injection of dried horse serum) previously dissolved in 0·5 c.c. saline solution. To each tube of the second series was added 0·5 c.c. of a 10-per-cent. solution of trichloracetic acid. The precipitates were read after 48 hours, as shown in Table I.

Table I.—Primary Interactions. Experiment I.

No. of tube.	Grammes of dried horse serum in each tube.	Dilutions of dried horse serum in 0·5 c.c. saline.	Precipitates in tubes to which 0·01 gramme dried antiserum was added.	Precipitates in tubes to which 0·5 c.c. of 10-per-cent. trichloracetic acid was added.
			mm.	mm.
1	0·05	1 in 10	1	60
2	0·005	1 „ 100	1·5	21
3	0·0005	1 „ 1,000	1·5	4
4	0·00005	1 „ 10,000	1·5	1·5
5	0·000005	1 „ 100,000	0·5	None
6	0·0000005	1 „ 1,000,000	0·3	None
7	0·00000005	1 „ 10,000,000	0·3	None
8	Control	Saline alone.	None.	—

Hence, in Tubes Nos. 5, 6, and 7 definite small precipitates are given by precipitin reactions in which the total amount of homologous proteid is insufficient to yield any visible precipitate when acted on by trichloracetic acid. Salicyl sulphonic acid, hydroferrocyanic acid, picric acid, mercuric chloride, and heat also failed to demonstrate the presence of 0·000005 gramme dried serum in 0·5 c.c. saline. Similar observations have already been recorded, notably by Linossier and Lemoine, as indicating the delicacy of the precipitin test for minute traces of homologous proteid, but we are not aware that the inference that the antiserum must be the main source of the substance that goes to form the precipitate has previously been made.

It is also noteworthy that the maximum precipitate is given by an amount of homologous proteid which, as will next be shown, is adequate to neutralise all (or practically all) the precipitin, and that no further increase of the homologous proteid will augment that precipitate. In view of the phenomena that we have yet to describe, we should therefore regard a precipitate of about 1·5 mm. as representing the precipitable content of 0·01 gramme of this particular antiserum. And, roughly speaking, since a precipitate of 1·5 mm. is given by trichloracetic acid with 0·00005 serum, we may also say that the weight of "precipitable" substance in 0·01 gramme of this antiserum may be represented by a figure of the same order of magnitude as 0·00005 gramme, in other words, that one part of this antiserum contains 0·00*x*. part of "precipitable" substance.

If after 48 hours one-fifth part of the clear superfluid from each precipitin reaction be transferred to each of two clean tubes, A and B, and if one series, A, be tested for residual precipitin by the addition of 0·0001 gramme dried horse serum in 0·5 c.c. saline, while the other series, B, is searched for residual horse serum by addition of 0·01 gramme dried antiserum in 0·5 c.c. saline, the following secondary interactions occur, the precipitates being read in 48 hours, as shown in Table II:—

Table II.—Secondary Interactions. Experiment I.

No.	Precipitates in A series (+ horse serum).	Precipitates in B series (+ antiserum).
	mm.	mm.
1	Not tested	Not tested.
2	None	2·5
3	None	1·5
4	0·3	0·5
5	0·5	0·3
6	0·5	0·3
7	0·5	0·3
8	0·5	None

These results show that the so-called precipitin was completely neutralised in Tubes Nos. 2 and 3, partially neutralised in Tube No. 4, and not noticeably diminished in Tubes Nos. 5, 6, and 7, when the precipita are compared with that given in the control Tube No. 8. The homologous proteid, on the contrary, was not exhausted in any tube, and in spite of the facts that only one-fifth of the amount originally present was taken, and that it had already reacted with antiserum to yield definite precipita, it was still capable of eliciting from a second supply of 0·01 gramme antiserum precipitates not less than those obtained in the primary interactions. An apparent exception is Tube No. 4, in which occurred a deposit much smaller than that primarily given. But the smaller precipitate in this instance reinforces our argument, since it is in all probability attributable not to exhaustion of the homologous proteid, but to the fact that the amount of horse serum (0·00005 gramme) originally present was just insufficient to neutralise 0·01 gramme antiserum, and that the amount actually present in the secondary reaction was 0·00001 gramme (already once acted on), making the interacting quantities more nearly comparable to those in the primary Tube No. 5.

In Tubes Nos. 4, 5, 6, and 7 uncombined precipitin coexisted with uncombined homologous proteid in the clear superfluids, an observation repeatedly made by Linossier and Lemoine, Eisenberg, Ascoli, and others.

Assuming for the moment that our two main conclusions were correct, (1) that the precipitum is derived mainly from the antiserum, and (2) that the homologous proteid is relatively not exhausted in any interaction, we were naturally led to investigate the effect of adding to minute quantities of the homologous proteid amounts of precipitin antiserum larger than those used in Experiment I. The results were confirmatory of our hypotheses.

Experiment II.—Taking an amount of dried horse serum (0·000005 gramme), sufficiently minute to escape detection by trichloracetic acid and other proteid precipitants (*cf.* Tube No. 5, Experiment I), we allowed it to interact with increasing amounts (0·01 gramme, 0·05 gramme, 0·1 gramme) of dried horse antiserum, the salt solution in each tube being made up to 1·1 c.c. The results are summarised in Table III.

Table III.—Experiment II.

No.	Amount of dried horse serum in grammes.	Amount of dried antiserum in grammes.	Precipita in tubes to which antiserum was added.	Precipita obtainable by ordinary proteid precipitants.
			mm.	
1	0·000005	0·01	0·3	Inappreciable
2	0·000005	0·05	1	"
3	0·000005	0·1	2	"

These results indicate that by increasing the amount of precipitin antiserum there may be obtained precipitates that are enormous relatively to those obtainable from the total amount of homologous proteid in solution. In view of these observations it becomes still more difficult to explain the origin of the precipitate otherwise than from the antiserum.

When similar observations are carried out with antisera for egg albumins, the results are in accordance with those obtained for horse serum and antiserum.

Experiment III.—Two corresponding series of tubes were prepared, as in Experiment I, containing from 0·0005 to 0·00000005 gramme dried hen egg white in 0·5 c.c. salt solution. To each tube of the first series and to a control was added 0·01 gramme dried antiserum (prepared in a rabbit by injection of dried egg white) previously dissolved in 0·5 c.c. salt solution. To each tube of the second series was added 0·5 c.c. of 10-per-cent. trichloracetic acid. The precipitates were read after 48 hours, as shown in Table IV.

Table IV.—Primary Interactions. Experiment III.

No.	Amount of dried egg white in grammes.	Dilutions of dried egg white in 0·5 c.c. saline.	Precipita in tubes to which 0·01 gramme dried antiserum was was added.	Precipita in tubes to which 0·5 c.c. 10-per-cent. trichloracetic acid was added.
			mm.	mm.
1	0·0005	1 in 1,000	1·5	5
2	0·00005	1 „ 10,000	1·5	1·5
3	0·000005	1 „ 100,000	1	Minute trace
4	0·0000005	1 „ 1,000,000	0·3	None
5	0·00000005	1 „ 10,000,000	0·3	None
6	Control	Saline alone	None	—

Again it appears that definite small precipita are obtainable by the interaction with precipitin antiserum of amounts of homologous proteid inadequate to yield a trace of deposit when thrown out of solution by trichloracetic acid.

After 48 hours one-fifth part of the clear superfluid from each precipitin reaction was transferred to each of two clean tubes, A and B. Series A was tested for residual precipitin by the addition of 0·1 c.c. of 1-per-cent. fresh egg white in salt solution (equivalent to 0·00012 gramme dried) to each tube, while in series B the presence of homologous proteid in excess was revealed by additions of 0·01 gramme dried antiserum in 0·1 c.c. saline. The total fluid in each tube was made up to 0·6 c.c. by addition of salt solution. The precipitates in these secondary reactions were read in 48 hours, as in Table V.

Table V.—Secondary Interactions. Experiment III.

No.	Precipita in A series (+ egg white).	Precipita in B series (+ antiserum).
	mm.	mm.
1	0	2
2	0	2
3	0·5	0·5
4	1	0·3
5	1	0·3
6	1	None

Among the data afforded by this experiment may be indicated (1) the complete neutralisation of the precipitin in the first two tubes only, and the coincidence of this point with the maximum precipitate obtainable in the primary interactions from 0·01 gramme of this antiserum; (2) the inappreciable exhaustion of the homologous proteid as a result of the primary interactions; and (3) the coexistence of uncombined egg white and its antiserum in the clear superfluids after the primary interactions are completed.

When larger amounts of egg antiserum were employed, correspondingly larger precipita were obtained, the masses of which were still more strikingly incompatible with an origin from the homologous proteid available.

Experiment IV.—To two tubes, each containing 1 c.c. of fluid antiserum (approximately equivalent to 0·1 gramme dried) prepared by injection of fluid egg white, there was added 0·1 c.c. of 10-per-cent. fresh egg white in salt solution (equivalent to 0·0012 gramme dried) and 0·1 c.c. of 1-per-cent. fresh egg white in saline (0·00012 gramme dried) respectively. The precipitates were read in 48 hours, with the results shown in Table VI.

Table VI.—Primary Interactions. Experiment IV.

No.	Amount of egg white in grammes.	Amount of antiserum in grammes.	Precipita in 48 hours.
			mm.
1	0·0012	0·1	7 (compact)
2	0·00012	0·1	11 (looser)

The precipitate in Tube No. 2 decreased to 9 mm. on the third day, and, though kept under observation up to the seventh day, it did not shrink below 8·5 mm. The same amount of egg albumin (0·00012 gramme dried) when precipitated by trichloracetic acid would yield a deposit not exceeding

2·5 mm. Hence the precipitum resulting from the precipitin reaction, notwithstanding its looser consistence, cannot be explained as a derivative of the homologous proteid alone.

After 48 hours 0·1 c.c. of the clear superfluid from each tube was transferred to each of two clean tubes, A and B. To each A tube was added 0·00012 gramme egg white in 0·5 c.c. saline; to each B tube 0·01 gramme antiserum in 0·5 c.c. saline. The precipitates in 48 hours are given in Table VII.

Table VII.—Secondary Interactions. Experiment IV.

No.	Precipita in A series (+ egg white).	Precipita in B series (+ antiserum).
	mm.	mm.
1	0·5	1·5
2	1·5	0·5

Therefore, both precipitin and homologous proteid coexisted uncombined in the clear superfluids; the precipitin was largely neutralised in the first tube, but not much in the second; the homologous proteid was not noticeably exhausted in any tube. The last result is the most important for our present purpose.

Tertiary Interactions.—Having noted that the secondary interactions invariably revealed the presence of homologous proteid not noticeably exhausted, whether the proteid were of the nature of egg white or of the nature of serum, we submitted some of the superfluids of the secondary interactions to the influence of a fresh supply of proteid and of antiserum. The addition of proteid revealed the presence or absence of precipitin according as it had not or had previously been neutralised. The addition of antiserum invariably revealed the presence of unexhausted homologous proteid by the deposition of precipitates which were not noticeably smaller than if the same quantities had interacted originally. It is unnecessary to detail these observations, since similar results are more strikingly displayed in the following experiments:—

Exhaustion Experiments.—These observations were so unexpected and so much at variance with accepted views regarding the precipitation of the homologous proteid that we instituted a series of experiments to try if, by successive additions of antiserum to the clear superfluids remaining after each interaction, we could exhaust the homologous proteid. As the following experiments show, we are not in a position to say whether the proteid is or is not appreciably used up as a result of interaction with precipitin antisera. Yet we have no conclusive evidence that it is so used up, and we have definite evidence that it is not entirely removed by any number of interactions or amounts of precipitin antiserum with which it has been tested.

Experiment V. First Interaction.—To 0·5 c.c. of 1-per-cent. fresh egg white in salt solution (equivalent to 0·0006 gramme dried) there was added 4·5 c.c. salt solution, and then 0·01 gramme dried antiserum (prepared in a rabbit by repeated injections of fluid egg white) in 0·2 c.c. saline. After 48 hours there was a deposit measuring between 0·5 mm. and 1 mm., and some flocculi in suspension.

Second Interaction.—The superfluid was then filtered, and to 4 c.c. of the filtrate was again added 0·01 gramme antiserum in 0·2 c.c. saline. In 48 hours the precipitate again exceeded 0·5 mm., and there was also some suspended matter.

Third Interaction.—The process was repeated, and 3 c.c. of filtrate plus 0·01 gramme antiserum yielded a precipitate about 1 mm. in 48 hours.

Fourth Interaction.—2·5 c.c. filtrate plus 0·01 gramme antiserum gave in 48 hours a large precipitate of 2·5 mm.

Fifth Interaction.—1·5 c.c. filtrate plus 0·01 gramme antiserum gave in 72 hours a 2-mm. precipitate.

Sixth Interaction.—The filtrate, measuring 1 c.c., was now divided equally between two tubes, A and B. To tube A was added 0·1 c.c. of 1-per-cent. fresh egg white in salt solution (0·00012 gramme dried), to tube B 0·01 gramme antiserum as before. After 48 hours the precipitates read in tube A 0·5 mm., in tube B 2 mm.

The first four interactions took place in tubes of about 5 mm. diameter, the others in tubes of 4 mm. diameter.

The smaller precipitates in the earlier interactions were probably due partly to the longer column of fluid through which they had to settle, and partly to the greater dilution of the precipitin antiserum retarding the reaction and rendering it not quite complete even in 48 hours.

The fluids did not altogether escape bacterial contamination, but up to the final interaction there was never more than a faint cloud beneath the aërial surface of the fluid. There was never any surface scum or pellicle, or any bacterial clouding in the deeper parts of the tube. The precipitates were distinctly flocculent, and could not have been mistaken for bacterial deposits. These remarks also apply to the other exhaustion experiments to be later described.

Notwithstanding, therefore, the progressive diminution of the available homologous proteid at each stage and the successive addiments of antiserum, the result is to show that in the final tube A there was no great residue of precipitin, whereas in tube B there was still sufficient homologous proteid to give the maximum precipitate from 0·01 gramme of this antiserum. This amount of homologous proteid could not exceed one-tenth of that orginally present, that is, it could not exceed 0·00006 gramme, and had already been acted on five times. These results are summarised in Table VIII:—

Table VIII.—Experiment V.

No. of interaction.	Amount of egg white remaining at each stage.	Total amount of fluid at each stage.	Amount in grammes of antiserum added at each stage.	Duration of each interaction.	Precipita at end of each stage.
	gramme.	c.c.		hrs.	mm.
1	0·0006	5	0·01	48	0·5+
2	0 00048 (once acted on)	4	0·01	48	0·5+
3	0·00036 (twice acted on)	3	0·01	48	1
4	0·0003 (three times acted on)...	2·5	0·01	48	2·5
5	0·00018 (four times acted on) ...	1·5	0·01	72	2
6	0·00006 (five times acted on) ...	0·5	0·01	48	2

In *Experiment VI* a much smaller amount (equivalent to 0·000000012 gramme dried) of egg white was originally taken and was allowed to react with 0·01 gramme dried antiserum (prepared in a rabbit with dried egg white). At the end of 48 hours the precipitum was noted, the superfluid was filtered, and a fresh supply of 0·01 gramme antiserum added. This process was repeated again and again, as summarised in Table IX. The precipitates were distinctly flocculent:—

Table IX.—Experiment VI.

No. of interaction.	Amount in grammes of egg-white remaining at each stage.	Amount of fluid at each stage.	Grammes of antiserum added at each stage.	Duration of each interaction.	Precipita at end of each interaction.
		c.c.		hrs.	mm.
1	0·000000012	5	0·01	72	slight = 0·3
2	0·0000000096 (once acted on)	4	0·01	48	„ = 0·3
3	0·0000000072 (twice acted on)	3	0·01	48	„ = 0·3
4	0·0000000048 (three times acted on)	2	0·01	72	„ = 0·3
5	0·000000003 (four times acted on)	1·25	0·01	48	more abundant = 0·5
6	0·0000000018 (five times acted on)	0·75	0·01	48	„ = 1

We omitted to test the superfluid of the final interaction with a relatively large amount of egg white, but, doubtless, had we done so, a large residuum of uncombined precipitin would have been revealed. The important fact, however, remains, that the homologous proteid, so far from showing evidence of exhaustion, yields more abundant precipitates at the end than at the beginning of the series, possibly owing to the increasing concentration of the precipitin.

In the next experiment a similar process of repeated interaction with antiserum was applied concurrently to three tubes containing different amounts of the homologous proteid.

Experiment VII.—The original amounts of fluid egg white were equivalent in Tube No. 1 to 0·000006 gramme, in Tube No. 2 to 0·000012 gramme, in Tube No. 3 to 0·000024 gramme. Of dried antiserum (prepared by injection of fluid egg white) 0·01 gramme was added to each tube, and the same dose was repeated at each successive interaction. The amount of fluid in the original tubes was made up to 1·1 c.c. with salt solution. At each stage the clear superfluids were transferred by means of a pipette to clean tubes. The amounts were not accurately measured, but about 0·2 c.c. of the fluid was left behind each time. The tubes were 4 mm. in diameter. The results are summarised in Table X:—

Table X.—Experiment VII.

No. of interaction.	Tube No.	Duration of each interaction.	Precipita at end of each stage.
		hrs.	mm.
1	1	48	1
	2	24	1
	3	24	1
2	1	48	1·5
	2	24	1·5
	3	24	1·5
3	1	48	1·5
	2	72	1·5
	3	72	1·5
4	1	48	0·3
	2	48	0·3
	3	48	0·5

At the conclusion of the fourth interaction the clear superfluids were removed as before, and an amount of fresh egg white (equivalent to 0·00024 gramme dried) was added to each. Within 48 hours a large precipitate between 2·5 and 3 mm. appeared in each tube, indicating a considerable excess of uncombined precipitin.

This experiment is somewhat unsatisfactory, for two reasons—the time intervals were not adjusted to ensure completion of each interaction, and the amount of superfluid removed at each stage was not accurately determined. We would not have recorded it at all, were it not for the fact that it is the only indication we have met that the homologous proteid may be diminished as a result of interaction with its precipitin. Even so it is not until after the third interaction that any diminution of homologous proteid is indicated, and we are unable to decide whether that is due to the proteid having been used up to any appreciable extent, or to its amount having been so reduced by successive subdivisions that it is no longer sufficient to produce the full precipitate from 0·01 gramme of this antiserum.

The cumulative evidence of these and other experiments, all of which give concordant results, renders it increasingly difficult to trace the precipitum to an origin other than the antiserum itself. Presumably the "precipitable" substance in the antiserum is the body commonly known as the precipitin. In any interaction, however, it is the homologous proteid that in some way renders the precipitin insoluble, and this it does without sensibly diminishing its capacity for further effective interaction with fresh precipitin.

Comparison of Antisera Prepared in the Rabbit with Natural Rabbit Serum.

Being led by our observations to the conclusion that an antiserum, prepared in a rabbit by repeated injections of some alien proteid, contained a substance or substances in sufficient abundance to yield definite precipita when thrown out of solution by interaction with the homologous proteid, which substance or substances the natural serum of the rabbit did not contain, we began a series of experiments to determine whether or not it were possible to obtain evidence of other differences between antisera and natural sera from the rabbit besides those revealed by precipitin tests.

Table XI.

Weight in grammes of 5 c.c. of natural rabbit sera (when dried).	Average weight.	Weight in grammes of 5 c.c. of antisera prepared in rabbit (when dried).	Average weight.
	gramme.		gramme.
0·4937	0·446	0·5273 (ox serum)	0·501
0·4681		0·5174 (horse serum)	
0·4471		0·5118 (ostrich egg I)	
0·4228		0·4980 (ostrich egg II)	
0·3998		0·4878 (hen egg I)	
		0·4675 (hen egg II)	

In the first place equal volumes of various antisera prepared in the rabbit and of the natural serum of several rabbits were dried to constant weight over calcium chloride at 37° C. *in vacuo,* and weighed. In several instances the mean of two concordant estimations was taken. The results are shown in Table XI.

The difference between the mean weight of antiserum and that of natural serum becomes all the more striking when it is borne in mind that most of the rabbits had lost weight during the process of immunisation, whereas the control rabbits were all in good condition.

We next examined by fractional heat coagulation two hen egg antisera prepared in the rabbit, and compared them under the same conditions with natural rabbit serum, with hen egg white, and with one of the above hen egg antisera after interaction with hen egg white. In the last case 1·3 c.c. of fluid hen egg antiserum reacted for 72 hours with 0·3 c.c. of 1-per-cent. hen egg white in normal salt solution and yielded an abundant precipitate. The clear superfluid was then removed, and treated in the same way as the other substances investigated.

The fluid antisera, sera, and egg white were diluted 20 times with normal salt solution and acidified to an equal degree with acetic acid. Some preliminary experiments were necessary to determine the most suitable quantity of the latter.

The results are given in the following table (XII), in which the lower figure of each pair presents the temperature in degrees centigrade at which opalescence was observed, the higher that at which flocculation and complete precipitation occurred. Clear filtrates were obtained after each heating, and from 30 to 40 minutes were allowed at each temperature to remove the proteid.

Table XII.

Hen egg antiserum.	Neutralised antiserum.	Rabbit serum.	Egg white.
59—62	53—60	56—60	59—62
		64—66	
68—70	68—70	69—70	68—70
71—72			
74—75	73—74	73—74	72—74
76—80	76—80	78—80	
84—86	84—86	82—84	

The figures indicate that in the antiserum there was present a body coagulating at 72° C., and disappearing after inaction with egg white. The precipitates that separated from the antiserum at this temperature and at

70° C. were dense flocculent masses, which settled to form large deposits. Such large deposits were not observed in the neutralised antiserum nor in natural serum at these temperatures. Further experiments on these lines are in progress.

The Precipitable Content and Precipitability of Antisera.

When a given amount (say 0·01 gramme) of antiserum is completely neutralised by its homologous proteid, so that no precipitin can be detected in the superfluid, then no further increase in the amount of homologous proteid will augment the precipitum, and that precipitum may be taken to represent the entire precipitable content of the given amount of that antiserum. Any reliable method of measuring the precipitum, such as that devised by Nuttall, would afford an indication of the precipitable content of an antiserum, provided that the amount of homologous proteid interacting were sufficient to neutralise all the precipitin within the limits of time allowed. As the complete neutralisation of the precipitin is not taken into consideration, and is not always secured by Nuttall's original method, we introduced a modification whereby complete neutralisation of the precipitin is secured by interaction with larger amounts of the homologous proteid. The bulk of the precipitate measured in this way affords a datum by means of which the precipitable content of different antisera may be compared.

Numerous observations serve to show that the precipitum is of a proteid nature, and, in discussing the primary interactions in Experiment I, we indicated another way in which the precipitable content may roughly be expressed—in terms of the amount of proteid to which the maximum precipitum from a given amount of antiserum is approximately equivalent. Thus we saw that the precipitable content of 0·01 gramme of the horse antiserum of Experiment I was 0·0000x gramme. The precipitable content of the same amount of the egg antiserum of Experiment III might be similarly represented by a figure of the same order of magnitude.

Estimated by our modification of Nuttall's method, the precipitum from 0·01 gramme of the horse antiserum of Experiment I, and of the egg antiserum of Experiment III, measured in each instance 0·024 c.c. In the common acceptation of the term these antisera would rank as equally "powerful," that is, capable of throwing out of solution equal amounts of their respective homologous proteids. We should prefer to say that the precipitable content of the two antisera was equal.

Yet there is a striking difference between them, unrevealed by Nuttall's method or by our modification, for the amount of the homologous proteid sufficient to neutralise all the precipitin in 0·01 gramme of antiserum is, roughly speaking, ten times greater in the case of the horse antiserum (0·0005 gramme, *cf.* Tube No. 3, Experiment I) than in the case of the egg antiserum (0·00005 gramme, *cf.* Tube No. 2, Experiment III). These data are not to be taken as accurately determining the minimum neutralising amounts of homologous proteid for 0·01 gramme of the antisera, as the intervals above and below the critical points are too great. Nevertheless they serve the purpose of indicating the nature of the difference between the two antisera in question. They show that the precipitable content of the egg antiserum is more easily discharged than that of the horse antiserum, since it requires less of the homologous proteid for complete neutralisation and precipitation.

In estimating the capacity of an antiserum for interaction with its homologous proteid, two factors, therefore, have to be considered: (1) the precipitable content of the antiserum, and (2) its precipitability. So far as our observations go, these factors appear to be mutually independent.

The results of operating with antisera whose precipitable content is not easily discharged are of interest, since they indicate how erroneous impressions may arise regarding the precipitation of the homologous proteid. In such cases the precipitates may show a progressive increase with each increase of the homologous proteid and only small precipitates with small amounts of the proteid.

In the readjustment of atom complexes that may take place within the animal body when its proteid molecules repeatedly encounter alien proteid molecules, as in processes of immunisation, it is not unreasonable to suppose that the reaction on the part of the host results in the formation of precipitable molecules which are endowed in certain circumstances with many, in other circumstances with few, atom complexes capable of interacting with corresponding atom complexes of the homologous proteid molecule. It is possible that the precipitable content of such an antiserum is determined by the number of precipitable molecules in a given molecule of the antiserum, while its precipitability is conditioned by the number of susceptible atom complexes that each molecule possesses.

The Rôle *of the Homologous Proteid in Precipitin Reactions.*

Our observations do not enable us to decide whether the homologous proteid is or is not diminished as a result of interaction with precipitin antisera. We have, however, definite evidence that it is not wholly exhausted and no conclusive evidence that it is sensibly exhausted even after repeated interactions with 0·01 gramme or single interactions with 0·1 gramme of antiserum, however minute the original amount of proteid may have been.

That the interaction is of a chemical nature we have no reason to doubt. That a given amount of antiserum will remove from solution definite amounts of its homologous proteid we may accept on the understanding that the amounts of proteid so removed are exceedingly minute. But that is not what is usually meant when it is said that the homologous proteid combines quantitatively with the precipitin to form the precipitum. What is meant is that measurable amounts of both substances disappear from the superfluid after each interaction. That this is true of the substance contributed by the antiserum we have abundant evidence; that it is true of the homologous proteid we have no evidence whatever.

Consistently with our observations, therefore, the results of a precipitin interaction cannot be stated in the form of a simple chemical equation. We are not in a position to formulate any definite hypothesis, but we may at least infer that the processes underlying the interaction are of a more complex nature. From the fact that the homologous proteid is not appreciably diminished, it is suggested that the proteid may exert a catalytic effect on the precipitable substance of the antiserum, whereby a molecular rearrangement is induced and the substance is thrown out of solution.

Conclusions.

1. The homologous proteid is not wholly removed from the superfluid of a precipitin interaction, whether it is more than sufficient or less than sufficient to neutralise all the precipitin present.
2. Conclusive evidence that the homologous proteid is sensibly diminished in similar circumstances has not been obtained.
3. The substance that is thrown out of solution is derived mainly from the antiserum.
4. The character of an antiserum depends upon two factors which are mutually independent: (1) the precipitable content; (2) its precipitability.
5. The precipitable content is indicated by the maximum precipitum obtainable from a given amount of the antiserum.
6. Its precipitability is indicated by the minimum amount of homologous proteid that will completely neutralise the precipitin in a given amount of the antiserum.
7. The solid content of precipitin antisera is increased relatively to that of natural sera.

We desire to express our indebtedness to Professor Anderson Stuart, in whose laboratory most of our work has been done, and to Dr. G. H. F. Nuttall, whose book has been of great service in giving an account of the work of other observers whose original communications we have been unable to consult.

Observations on the Development of Ornithorhynchus.

By J. T. Wilson, M.B., Professor of Anatomy, and J. P. Hill, D.Sc., Lecturer on Embryology, University of Sydney, N.S.W.

(Communicated by Sir William Turner, K.C.B., F.R.S. Received December 5,—Read December 14, 1905.)

(Abstract.)

The paper treats of certain stages in the intrauterine development of the egg of Ornithorhynchus.

The stages dealt with include the following:—

(1) An early (eight-celled) stage of segmentation.

(2) A stage manifesting a later phase of the segmentation-process.

(3) A stage of early germinal-layer formation, in which the cellular blastoderm is almost exclusively arranged in the form of a much-attenuated epithelial membrane covering part of the yolk-mass. The arrangement on the whole resembles that illustrated in fig. 22, Taf. 8, and fig. 33, Taf. 9, in R. Semon's work on "Monotreme Development."* Certain differences are, however, noticeable, more especially as regards the absence of the deeply placed nuclei figured by Semon.

(4) A stage of more advanced germinal-layer formation, characterised by the complete differentiation of the yolk-entoderm and of a lenticular mass of cells connected with the outer layer of the vesicle, which would seem to represent an early condition of "primitive knot" formation. This stage represents that of the completed "first phase of gastrulation."

(5) A stage exhibiting the characteristics of the "second phase of gastrulation." These include the presence of a fully-developed "primitive knot" which may henceforth be designated as "archenteric," owing to the formation in connection with it, by invagination, of an "archenteric," or "gastrulation-cavity." Meanwhile there has also developed—quite independently of, and

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* 'Zool. Forschungsreisen in Australien,' etc., 1894, Bd. 2, Lief. 1.

remote from, the primitive or archenteric knot—a primitive streak, of typically mammalian character.

(6) A stage which, for convenience of classification, is termed by the authors the "postgastrular." This is characterised, *inter alia*, by the presence of a greatly elongated area of axial differentiation consisting of a primitive streak, together with a "Hensen's knot" and a long "archenteric plate," the two latter structures being derivatives of the "primitive" or "archenteric" knot of the preceding period. The archenteric plate ends anteriorly in an expanded "protochordal-plate" segment.

In this stage the primitive streak has practically retained its earlier form and dimensions, but it is now contiguous to, and has the appearance of being continuous with, the posterior end of the thickening of "Hensen's" (archenteric) knot.

The "archenteric plate" is derived from the dorsal wall of a greatly elongated archenteric canal (*i.e.*, the archenteric invagination-cavity of the preceding stage). From this archenteric plate are derived both the chorda-Anlage and also, laterally to this, a small amount of "gastral" mesoderm.

(7) A "neurular" stage is described, in which the medullary plate is present as a completely flattened and spatulate structure. This is expanded, in the head-region, into a wider "cephalic plate." This again is divisible into two divisions which seem to correspond to the "archencephalon" and the "deuteroencephalon," respectively, of von Kupffer.

The cephalic plate further exhibits a series of neuromeric segments, all of which, with the exception of the most anterior, belong to the region of the "deuteroencephalon." One neuromere, on account of its relations to the acustico-facial ganglion, is designated as "facial." In front of this neuromere at least five "prefacial" neural segments are met with, whilst behind the same "facial" neuromere three definite "postfacial" neuromeres are encountered.

In the posterior region of the cephalic portion of the medullary plate, indications are to be found, in the case of slightly younger specimens, of the existence of additional "postfacial" segments behind the persistent 3rd postfacial neuromere.

Laterally from the margins of the cephalic medullary plate there are found, on each side, three ganglionic plate-like expansions of the "neural crest." These represent the rudiments of the trigeminal, acustico-facial, and vago-glossopharyngeal ganglia.

The relations of these ganglionic plates with the various neuromeres are as follows:—The facial neuromere is connected with the "pedicle" of the acustico-facial ganglion, which at this period consists of a tract of cellular tissue. Of the three definite postfacial neuromeres the 1st or most anterior has no recognisable ganglionic connection at the stages examined. The 2nd and 3rd postfacial neuromeres are connected laterally with the vago-glosso-pharyngeal ganglionic plate. Of the five prefacial neuromeres, the 1st, *i.e.*, the one immediately in front of the facial neuromere, has at no time any ganglionic connection, and lies opposite the hiatus between the trigeminal and the acustico-facial ganglionic plates. The next succeeding neuromeres in front of the 1st, *i.e.*, the 2nd to the 5th prefacial neuromeres, are each connected laterally with the broad basal portion, or wide "pedicle," of the trigeminal ganglionic plate.

The following points of more special interest amongst those set forth in the extended paper may here be specified.

(*a*) The very early differentiation of the layer of yolk-entoderm surrounding the yolk-mass of the monotreme egg.

(*b*) The original entire independence of the primitive streak from the primitive knot and its "gastrulation-cavity."

(*c*) The subsequent intimate approximation of these structures.

(*d*) The early appearance of an area of special differentiation in the vicinity of the primitive streak, in the early blastoderm; and the later conversion of this "primitive-streak-area" into an "embryonic area" proper, by the annexation of the region surrounding the "primitive" or "archenteric" knot.

(*e*) The precise mode of disappearance of the ventral wall, or floor, of the archenteric or invagination-cavity.

(*f*) The occurrence of peculiar segmental cell-masses in the substance of the "primitive knot," where that constitutes the parietes of an archenteric canal or its representative.

(*g*) The diagrammatically clear demonstration of various features of neural development, including: the well-marked neuromeric segmentation of the cephalic region of the flattened medullary plate; the differentiation of early plate-like ganglionic expansions of the neural crest in the cephalic region; the presence of various cellular connections between the cephalic ganglionic plates and certain of the neuromeric segments of the medullary plate.

(*h*) The relative insignificance of the "archencephalic" subdivision of the cephalic portion of the medullary plate, from which the fore-brain and most, if not all, of the mid-brain, are derived.

Remarks on Mr. Plimmer's Note on the Effects produced in Rats by the Trypanosomata of Gambian Fever and Sleeping Sickness.

By H. Wolferstan Thomas, M.D., C.M. (McGill) (J. H. Todd Memorial Fellow in Tropical Medicine), Liverpool School of Tropical Medicine, Johnston Tropical Laboratory, University, Liverpool.

(Communicated by Professor R. Boyce, F.R.S. Received April 8,—Read May 11, 1905.)

Mr. Plimmer reports observations* on three rats inoculated with trypanosomes from a monkey infected with the parasite of Uganda Sleeping Sickness and on 14 rats infected with the trypanosoma of Gambian Fever. The three rats inoculated with Uganda Sleeping Sickness never exhibited organisms in their blood, but after six to nine months developed paresis ending in death.

Post-mortem.—No macroscopic lesions of any organ were found. Examination of the blood gave negative results, as also did extracts of the organs, except that of the spinal cord, in which a few trypanosomes were found. Animals inoculated with some of this spinal cord extract did not show parasites in their blood. The rats infected with the Gambian Fever trypanosoma showed parasites in their blood and died.

Mr. Plimmer concludes from these experiments that Gambian Fever and Uganda Sleeping Sickness are distinct diseases.

My colleague, Dr. Linton, and myself compared the animal reactions of the trypanosomes derived from Uganda and Congo Free State Sleeping Sickness cases, and Uganda and Congo Free State trypanosome fever cases, with those of the parasites obtained by Drs. Dutton and Todd from three cases of natives suffering from Gambian Fever.

I have had the opportunity of using the same strain of Uganda Sleeping Sickness parasite as Mr. Plimmer, and he has experimented with one of the strains of trypanosomes from the Gambia Fever cases.

I have been able to compare 10 strains derived from Congo Free State Sleeping Sickness cases which were sent me by the members of our Congo Free State Expedition. In addition I have had four strains of Congo Native

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* H. G. Plimmer, F.L.S., "Note on the Effect produced on Rats by the Trypanosomata of Gambia Fever and Sleeping Sickness," communicated by Dr. C. J. Martin, F.R.S., received December 1, 1904, read January 19, 1905.—'Roy. Soc. Proc.,' No. 504, February 24, 1905, vol. 74, pp. 388—390.

Fever parasites, and since May, 1904, three strains of trypanosomes obtained by me from cases of Sleeping Sickness which died in Liverpool.

I have been able to inoculate rats directly with cerebro-spinal fluid from my cases. I have, therefore, been able to compare the trypanosomes derived from many different strains.

After a comparison of over 1000 experiments, nearly 600 being on rats, I have had to conclude that all the parasites are identical with *T. gambiense.* Rats inoculated with a Sleeping Sickness strain show parasites in their blood and finally succumb to the infection in the same way as do their controls infected with a Gambian Fever strain. No symptoms of paraplegia have been observed in any of the rats. *Post-mortem.*—The same enlargement of the spleen and some enlargement of the glands are observable.

Mr. Plimmer reports that morphologically the parasite of Gambian Fever differs from that of Sleeping Sickness in similar animals, the former being larger and more easily stainable than the stumpy, badly staining trypanosomes with large vacuoles of Sleeping Sickness.

I am not in accord with these observations, after numerous observations in parallel series with each strain of trypanosome, *i.e.*, same species of animal and same number of passages, and passage through same species of animals. I can detect no marked difference. Both stumpy and large forms are met with, vacuoles are most often observed in films made from very serous blood from exudates, and are often present in films which are allowed to dry slowly or are improperly fixed.

Experiments were made by inoculating rats and larger animals with the Gambian Fever trypanosomata, and, after the appearance of the parasite, injecting them with Uganda or Congo Sleeping Sickness organisms, and *vice versâ,* using controls in all cases. From a comparison of the blood of these animals with their controls, both in fresh and stained films it was impossible to determine which parasite was the Gambian one.

Professors Laveran and Mesnil, and the members of our Congo Expedition, from a comparison of the trypanosomes of Gambian Fever and Sleeping Sickness, have also been unable to detect any differences.

Professor Laveran, who has had the opportunity of working with Gambia Fever and Uganda Sleeping Sickness parasites, in a private communication which he allows me to make public, states that he has never observed symptoms of paralysis in his series of rats infected with these strains.

I, therefore, do not feel that Mr. Plimmer is justified in concluding that Gambia Fever and Sleeping Sickness are distinct diseases.

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The Mechanism of Carbon Assimilation in Green Plants: the Photolytic Decomposition of Carbon Dioxide in vitro.

By Francis L. Usher and J. H. Priestley, B.Sc.

(Communicated by Professor Morris W. Travers, F.R.S. Received April 30,—Read May 10, 1906.)

(From the Chemical and Botanical Departments, University College, Bristol.)

In a previous paper[*] it was shown that carbon dioxide is decomposed in the green parts of plants independently of vital or enzymic activity, formaldehyde and hydrogen peroxide being produced. It follows from the analysis of the process of carbon assimilation there set out that this first step, the photolysis of carbon dioxide, should be capable of being artificially induced under laboratory conditions.

It is impossible here to give even a brief account of the work of previous investigators on these lines; it is sufficient to state that all experiments with chlorophyll solutions have given negative results, and as regards those with other forms of chlorophyll, such as dried powdered leaves or expressed juice, the balance of evidence favours the view that no decomposition takes place. Reference may be made to papers by Friedel[†] and Macchiati[‡] on the positive side, and by Harroy,[§] Herzog,[||] and, quite recently, Bernard,[¶] who obtained only negative results. Experiments with uranium compounds will be considered later.

I. *Experiments on the Chlorophyll Catalysis of Carbon Dioxide.*

In view of the very perfect chemical and physical arrangements which assist the decomposition of carbon dioxide in the plant, it is obvious that attention must be paid to these points in any experiment performed outside it. It appears likely that the failure of so many attempts on the part of previous observers to carry this out has been due to a neglect of such considerations—not so much with regard to the chemical as the physical conditions. The chemical conditions are those which are necessary when any other highly reversible action is being dealt with, viz., that one at least of the products be rapidly removed from the sphere of action. The physical conditions were first enunciated by Timiriazeff, in the Croonian Lecture for 1903, and are as follows:—(1) The optical sensitiser must be in the form of an extremely thin film; and (2) the concentration of the sensitiser in this film must be so great as to ensure a large transformation of the incident solar energy within a very small space. The fulfilment of both these conditions in the case of chlorophyll in the plant is a matter of common knowledge, and the experiments to be described were designed to reproduce as far as possible the essential mechanical, physical, and chemical arrangements which obtain in the green leaf.

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[*] 'Roy. Soc. Proc.,' B, vol. 77, p. 369.

[†] 'Comptes Rendus,' 1901, vol. 132, p. 1138.

[‡] 'Bull. Soc. Bot. Ital.,' 1903.

[§] 'Comptes Rendus,' 1901, vol. 133, p. 890.

[||] 'Zeit. physiol. Chem.,' 1902, vol. 35, p. 459.

[¶] 'Comptes Rendus,' 1905, vol. 140, p. 509.

It should be mentioned here, as the result of some observations by one of us in conjunction with Miss Irving* on the structure of the large chloroplasts of *Selaginella* and *Chlorophytum*, that the chlorophyll is restricted to the exterior of the granule, and that the thickness of the film is about $2{\cdot}5 \times 10^{-3}$ mm. Whether this applies generally or not is of no consequence in this connection, the essential point being that we have here an actual working arrangement, and one which can be imitated without difficulty.

The greatest possible concentration of chlorophyll is obtained when the solvent used for extracting it has been completely driven off, and if material is employed which contains very little else that is soluble in the liquid in which the final solution of the chlorophyll takes place; one may regard the concentration of the chlorophyll thus obtained as being approximately the same as in the plant. For these experiments the material used was the leaves of grass or wheat, chosen on account of their comparative freedom from fats and oils. Alcohol was used for the extraction, and petroleum ether for the final solution of the chlorophyll.

In the first series of experiments pieces of glass plate, 5 × 4 inches, were covered with an aqueous solution of gelatine so as to form a layer 1 to 2 mm. thick. When set, this was painted over with a solution of chlorophyll in petroleum ether or benzene. In this way a fairly uniform film of chlorophyll is obtained, and if, when the plate is placed in carbon dioxide, light is allowed to strike it in the direction → carbon dioxide → chlorophyll → gelatine, we have a reproduction of the essential features of the arrangement in the living cell. Measurements of the thickness of the chlorophyll film gave an average value of about 6×10^{-3} mm.

On placing plates prepared in this manner in a bell jar containing moist carbon dioxide and exposing to light, the chlorophyll in the course of a few hours became completely bleached, and the gelatine developed a red colour when immersed in a solution of rosaniline decolourised with sulphurous acid. The experiment was repeated several times with the same result, and when performed on a larger scale the distillate from an aqueous solution of the gelatine was found to contain formaldehyde, the substance being identified by the characteristic methyleneaniline and tetrabromohexamethylenetetramine reactions. The melting point of the methyleneaniline was within 2° of that of the pure substance. It will be seen that the action which takes place under these conditions is the same as that which occurs in a green plant when the protoplasm has been killed and the enzymes destroyed; the accumulation of formaldehyde is possible so long as any chlorophyll remains to remove the hydrogen peroxide. The use of gelatine may be avoided by a modification of the arrangement just described. A layer of water in a shallow porcelain dish replaces the gelatine, and the chlorophyll film is obtained by dropping the petroleum ether solution over the surface from a pipette, at the same time directing a current of air against it so as to evaporate all the solvent before the chlorophyll can collect in patches. Experiments performed in this manner gave the same results as those with the gelatine films. The latter are more convenient to manipulate, but it is preferable to dispense with the gelatine if the object is to collect as much formaldehyde as possible, since this substance has a larger concentration in the residue than in the distillate.

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* At present unpublished.

A second series of experiments was undertaken with the object of ascertaining whether the process could be carried as far as the evolution of gaseous oxygen. It has already been pointed out in a previous paper that the decomposition of the hydrogen peroxide in the plant is brought about by an enzyme, probably a "catalase," and it was also shown that a certain amount of oxygen can be evolved from plants which have been killed, provided that the enzymes are not destroyed.

The catalase employed was obtained from sheep's liver by extracting the dried and powdered material with water containing a little thymol, the enzyme being precipitated by excess of absolute alcohol. The arrangement of the experiments was the same as in the first series, but the gelatine was made up with an aqueous solution of the catalase instead of with pure water, and the solution thus prepared was spread upon a strip of tinned iron 60 cm. long and 3 cm. wide. After painting it over with chlorophyll solution, the strip was placed inside a glass tube, sealed at one end, just large enough to contain it, and which was afterwards drawn out at the open end to a capillary, and sealed to a T-piece connecting it, through two stop-cocks, with a supply of carbon dioxide, and a pump. The carbon dioxide used was passed over red-hot copper in order to remove traces of oxygen, and the tube was exhausted and refilled six times. A similar tube without any catalase was treated in the same way. When the tubes had been filled with oxygen-free carbon dioxide for the sixth time, they were sealed off at the capillary portion and exposed to sunlight. In the course of an hour the gelatine in the tube containing the enzyme was swollen with bubbles of gas, and the chlorophyll film became distorted and broken up. The only visible change in the other tube was the bleaching of the chlorophyll. When the tubes had been exposed to light for two days they were attached to a Töpler pump by a short piece of rubber pressure tubing. After completely exhausting the system on the pump side of the capillary, the latter was broken off inside the pressure tubing, and the gas pumped out through a tube packed tightly with soda-lime, which absorbed all but a trace of the carbon dioxide. The tube containing the catalase yielded 0·6 c.c. of oxygen, with a trace of nitrogen, while the other gave a minute bubble of nitrogen only, the latter being derived in each case from the air present in the carbon dioxide before its passage over the red-hot copper. Two more similar experiments gave 2 c.c. and 1·2 c.c. of oxygen respectively.

It was found that if the exposure to light was continued until the chlorophyll was bleached, the enzyme was poisoned by the accumulating formaldehyde. This is precisely what occurs in the plant under similar conditions; that is, when the protoplasm only has been killed.

According to the analysis of the photosynthetic process given in the paper already referred to, where it was shown that the condensation of formaldehyde to carbohydrates is dependent upon the healthy condition of the protoplasm, one would expect non-chlorophyllous living cells to be able to bring about this change. It appears from the feeding experiments of Bokorny, Bouilhac, and Tréboux that light is necessary in the case of green plants, and we have found that this is also the case with the white petals of *Saxifraga Wallacei*, which are capable of forming starch in the light from a 0·001-per-cent. solution of formaldehyde, though no condensation takes place in the dark. In a further series of experiments these petals, when quite free from starch, were painted with chlorophyll solution, and floated on water charged with carbon dioxide in a bottle placed in the light. In the course of a day they were found to contain starch. In this case we have what is essentially a green leaf arranged in a different way, though it is, of course, a very inefficient substitute for the natural organ.

As it was of interest to ascertain whether formic acid is actually produced as an intermediate product in the photolytic reduction of carbonic acid to formaldehyde, an experiment similar to those described in Series I above was performed, the gelatine being dissolved in a solution of sodium bicarbonate. In this case the whole of the bicarbonate was converted into formate. When neutral or slightly acid gelatine was used, no formic acid could be detected. With very large concentrations of carbon dioxide, formic acid appears to be the sole product, since when sealed tubes containing chlorophyll, water, and liquid carbon dioxide were exposed to light, no formaldehyde was found, though a considerable quantity of formic acid was produced.

If, however, formic acid is an intermediate product in the photolysis of carbon dioxide in the plant, the latter should be able to build up carbohydrates from this substance. This was found to be the case with *Elodea*, which, when exposed to light in a 0·02-per-cent. solution of formic acid, gave off oxygen and formed starch; no starch was formed, or oxygen evolved, from *Elodea* kept in the dark, carbon dioxide being carefully excluded in each case. When the plant was previously killed, the same changes took place as when carbon dioxide was used, that is to say, the chlorophyll became bleached, and the plant was subsequently found to contain formaldehyde.

II. *The Photolytic Decomposition of Carbon Dioxide in the Presence of Uranium Compounds.*

In a previous paper, to which reference has been made above, it was stated that Bach's observations had been repeated and confirmed in the case of uranium acetate, minute quantities of formaldehyde being found in the distillate. Since then our attention has been called to a recent paper by Euler,* who was unable to confirm Bach's observations. A more detailed investigation has therefore been made, and, since it cannot be proved that any particular organic substance which may be produced is formed from the carbon dioxide, and not from the electro-negative portion of the organic salt when the latter is employed, a purely inorganic sensitiser has been used in the present series of experiments.

In the first of these, which was a repetition on a larger scale of one which has already been described, a large Pettenkofer tube was filled with a litre of 2-per-cent. uranium sulphate solution, and a slow current of carbon dioxide was passed through it, the whole apparatus being placed on the roof in order to utilise all the available sunlight. Within an hour of passing the carbon dioxide the contents of the tube were considerably turbid, and at the end of the exposure, which lasted from January 27 till February 9, the precipitate amounted to several grammes, and was of a pale violet colour. The greater part was soluble in acetic acid, and was found to be a mixture of uranous and ordinary uranic hydroxides. The insoluble residue* was a hydrate of uranium peroxide, being characterised by its insolubility in acetic acid, and also by the fact that it imparted a brownish-red colour to a solution of titanium oxide in sulphuric acid. The filtrate from the mixed hydroxides was distilled, and the distillate was examined for formaldehyde. None, however, was found, though the liquid energetically reduced Fehling's solution and silver nitrate. It was subsequently found to contain formic acid, the lead salt of which was prepared and identified. The undistilled residue was then evaporated down at 100°, and when nearly solid was repeatedly extracted with dry ether, in order to remove any formic acid which had not evaporated. The residual solid was extracted with absolute alcohol, and the solution on evaporation left a small quantity of a brown syrup, bitter to the taste, which reduced Fehling's solution. When treated with phenylhydrazine acetate no crystalline derivative could be separated, nor could the syrup itself be induced to crystallise. On account of its appearance, taste, and reducing action, it presents a striking resemblance to the substance obtained by Butlerow† from formaldehyde and milk of lime, and called by him "methylenitan." It has been shown that formaldehyde, when allowed to stand for some time in presence of uranic hydroxide, gives rise to a substance with similar properties. It does not appear possible to make any more explicit statement with regard to the identity of the substance produced from carbon dioxide in the manner described with methylenitan, since it is produced only in minute quantities, and does not admit of purification, or of the preparation of a derivative which can be purified; it is, however, worth while to notice that, as regards the two substances produced by the action of light on carbon dioxide in the presence of uranium sulphate on the one hand, and by the condensation of formaldehyde by uranic oxide on the other:

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* 'Ber. deut. chem. Ges.,' 1904, vol. 37, p. 3415.

(1) They are both organic substances which char on heating.
(2) Both are insoluble in dry ether, and soluble in water and alcohol.
(3) Both have a bitter taste, and reduce Fehling's solution.
(4) Neither reacts with phenylhydrazine.

Since it was conclusively proved by this experiment and those which preceded it that formic acid is one of the products of the action of sunlight on carbon dioxide, and in view of the fact that the quantity of any product formed is materially influenced by the concentration of the substance giving rise to it—which is necessarily small when that substance is carbon dioxide—it was thought that some light would be thrown on the reaction if one started with formic acid, instead of with carbon dioxide. For this purpose a solution of uranyl formate was employed, and the method has the advantage that any removal of formic acid from the system is indicated by a corresponding precipitation of uranic hydroxide. The uranyl formate for these experiments was prepared by heating uranyl nitrate (obtained from Kahlbaum) until oxides of nitrogen were no longer evolved, and dissolving the residual uranic oxide in the least possible quantity of 5-per-cent. formic acid. Preliminary experiments were carried out in order to ascertain whether any visible change takes place when the solution is exposed to sunlight. It was found that on placing a flask containing it in the light, the liquid became turbid after about 10 minutes' exposure, and in half an hour a considerable precipitate had accumulated. The precipitate at first produced is of a pure yellow colour, and consists of ordinary uranic hydroxide. After a short time it becomes violet-coloured, and, when a considerable quantity has collected, consists of a mixture of uranic and uranous hydroxides, together with small quantities of uranium peroxide. As in a large number of experiments no formaldehyde could be detected, several experiments were performed with a view to ascertain whether any gaseous products were formed. In one of these, a glass tube about 30 cm. long and 2 cm. bore was half filled with a solution of uranyl formate, drawn out to a capillary, exhausted, and sealed. After four days' exposure to sunlight the tube was connected with a Töpler pump and about 50 c.c. of gas were collected, which on analysis was found to consist of approximately equal volumes of carbon dioxide and hydrogen. This experiment was repeated several times, with the same result, though the carbon dioxide and hydrogen were not always produced in the same proportions. It should be stated that any uranium salt which remained in solution was invariably reduced to a green uranous compound, and this, no doubt, accounts for the variations observed in the relative volumes of the two gases.

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* UO_4 dissolves slowly in acetic acid, if uranous compounds are present.

† 'Comptes Rendus,' vol. 53, p. 145; 'Liebig's Annalen,' vol. 120, p. 296.

The foregoing experiments show that carbon dioxide in the presence of uranium compounds gives rise, under the influence of light, to formic acid, and that formic acid under the same conditions gives rise to carbon dioxide and hydrogen. It is probable, for reasons which will appear later, that we are dealing with several consecutive reactions which, under the conditions of the experiments, have widely different velocities, and which are not strictly reversible. If this be so, it follows that it will be very difficult, if not impossible, to isolate certain intermediate products. For example, in the case of the first series of experiments with uranium sulphate, it may be questioned whether the formic acid found really represents the first stage in the photolytic decomposition of carbon dioxide; it may quite possibly be the final product of a reaction between formaldehyde and one of the hydroxides present, following on the primary reaction which gave rise to the formaldehyde, and having a much higher velocity. In such a case, one could not expect to find formaldehyde in the final mixture. As the question at issue is whether or not formaldehyde is produced at any stage in the series of reactions which take place when sunlight acts on carbon dioxide under the conditions described, and as it was found that formaldehyde could not in any way be isolated from the mixture, it appeared that a careful study of the reactions of formic acid and aldehyde with such substances as were formed under the conditions of the experiments was the method most likely to settle the question. It may be worth while to point out here that a careful distinction must be maintained between those reactions which are endothermic, and derive the necessary energy from the sunlight, and those in which the action of light is purely catalytic.

Solutions of uranium salts—especially those like the formate—have always an acid reaction, and are probably hydrolysed to a considerable extent, so that any hydrogen peroxide that is formed is not completely removed from the system, but remains in equilibrium with the difficultly soluble uranium peroxide. We have, therefore, to consider reactions which may occur between

(i) Formic aldehyde *and* uranium peroxide.
(ii) „ acid „ „ „
(iii) „ „ „ hydrogen „
(iv) „ aldehyde „ „ „
(v) „ „ „ uranic oxide.

It has been shown by Geisow* that when hydrogen peroxide reacts with solutions of formaldehyde in neutral or acid solution, hydrogen and carbon dioxide are the sole products, no formic acid being produced. On the other hand, the peroxides of lead and manganese give hydrogen and a metallic formate. It has been found that uranium peroxide reacts in the dark with formaldehyde, and that hydrogen and uranium formate are produced, but no carbon dioxide. Hence, in such a system as the one under consideration, where there is a constant but very small concentration of hydrogen peroxide, there is the possibility of explaining the production of both hydrogen and carbon dioxide, the reaction taking place between the formaldehyde and hydrogen peroxide, though, of course, at the expense of the uranium peroxide and this appears to be the probable explanation, when we consider that the velocity of reaction in the case of hydrogen peroxide must be very much greater than in the case of uranium peroxide. The fact that hydrogen and carbon dioxide are formed as the result of the interaction of hydrogen peroxide and formaldehyde is a piece of evidence which strongly supports the view that the latter substance is formed in the experiments we are considering, provided that formic acid does not give rise to these gases under similar conditions. As no record of experiments on these lines could be found the point was investigated. The mixture of formic acid and hydrogen or uranium peroxide was placed in a Zeisel flask with a condenser attached. This was first connected with some bulbs containing lime-water, and a current of CO_2-free air was passed through the apparatus while the mixture was heated on a water-bath. Considerable quantities of carbon dioxide were evolved, as shown by the precipitation of calcium carbonate. A fresh quantity of the reaction-mixture was then taken, and the apparatus connected with a Schiff's nitrometer while a current of carbon dioxide was passed. Not a trace of hydrogen was evolved, although the mixture was boiled for three hours. There is apparently no difference between the reactions in the case of hydrogen peroxide and uranium peroxide, except that the latter proceeds more slowly. These experiments were carried out in the dark.

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* 'Ber. deut. chem. Ges.,' 1904, vol. 37, p. 515.

Experiments with ordinary uranic oxide showed that formaldehyde reduced it both in the dark and in the light, uranous oxide and a formate being produced, but only in the light was the violet colour observed. It appears that this coloration is merely a surface effect, since the precipitate, when dissolved in acid and reprecipitated, has the ordinary black colour of uranous oxide.

The following experiments bring out rather more clearly the distinction between formic acid and aldehyde mentioned above :—

To one of two tubes containing pure uranium peroxide, a dilute solution of formaldehyde was added; to the other, some dilute formic acid. After exposure to light from February 16 to February 19, no effect was visible in the tube which contained formic acid, whereas the other contained uranous formate and a violet-coloured precipitate. In order to examine the gases produced in the two cases, two more tubes were prepared, one of which (*a*) contained uranium peroxide, formic acid, and formaldehyde, while the other (*b*) contained uranium peroxide and formic acid only. Both were drawn out to a capillary, exhausted, and sealed. They were exposed to light from February 27 till March 6, when the gases were pumped out and examined: (*a*) contained 4·8 c.c. of carbon dioxide and 4·8 c.c. of hydrogen, while (*b*) contained 9·8 c.c. of carbon dioxide and no hydrogen.

There is thus no direct experimental evidence that formaldehyde is produced under these conditions, that is to say, it has not been isolated and identified, but there is indirect evidence of its formation at some stage. Whilst hydrogen is always found among the products of the action of light on carbon dioxide in the presence of uranium compounds, under no conditions in any way approaching those of the experiments in question has hydrogen been obtained from formic acid. The reduction of uranic oxide, or of uranium peroxide, to a violet-coloured lower oxide, which was always observed in the experiments with carbon dioxide, can again only be brought about by formaldehyde; whilst the formation of a substance with the properties of methylenitan, which has only been prepared from formaldehyde, furnishes additional support to this view.

Summary.

I.—(1) Photolytic decomposition of aqueous carbon dioxide can take place in the presence of chlorophyll, independently of vital or enzymic activity, provided that the necessary physical and chemical conditions are strictly adhered to.

(2) The products of the decomposition are formaldehyde and hydrogen peroxide, formic acid being an intermediate product.

(3) It is possible to reconstruct the process of photosynthesis outside the green plant, (*a*) as far as the production of formaldehyde and oxygen, by introducing a suitable catalysing enzyme into the system, and (*b*) as far as the production of oxygen and starch, by introducing, in addition to the enzyme, certain kinds of non-chlorophyllous living protoplasm.

II.—(1) There is direct experimental proof that formic acid is a product of the photolytic decomposition of carbon dioxide in the presence of an inorganic uranium salt.

(2) Formaldehyde has not been isolated and identified in the case of an inorganic uranium salt, but a study of the reactions involved favours the view that it is formed as a transitory intermediate product.

The Viscosity of the Blood.

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(Communicated by Professor Gotch, F.R.S. Received May 7,—Read May 31, 1906.)

The full importance of a knowledge of the variations in the viscous resistance to be overcome by the blood in circulating through the capillaries and smaller vessels, and the actual significance of such data to the more thorough consideration of a large number of normal and pathological conditions, especially those of the circulatory system, has perhaps never been fully realised or appreciated either by physiologists or by clinicians in this country.

Although the subject of the viscosity of the blood has from time to time during the last 60 years attracted the attention of a few well-known investigators, yet, so far as we know, there has been no serious attempt to apply systematically the results of their researches to either the theory or practice of medicine.

The possibility of such investigations proving factors of consequence in certain physiological and pathological states has been strongly emphasised by Professor Osler in the 'American Journal of the Medical Sciences,' writing on the subject of chronic cyanosis with polycythæmia. "It is especially important to test the viscosity of the blood by accurate physical methods, and to determine the relation of the number of corpuscles to the viscosity."

But before entering into any details of the present series of experiments, it may be well to give here a simple interpretation of the term viscosity.

When a liquid is flowing steadily through a tube or pipe, the layers of the fluid immediately next to the walls of the containing channel are practically at rest, and consequently act as a drag upon the more rapidly moving layers nearer the central stream. Now the force exerted by the sum total of these "drags," or shears, that is, the viscous resistance offered to the fluid motion, is different for different liquids. Or we may say briefly that the *viscosity of a liquid is that property whereby it resists the relative motion of its constituent parts.*

Historical.

From experiments upon the flow of distilled water through capillary glass tubes placed horizontally, Poiseuille, in 1843, obtained data which find full expression in the well-known formula—

$$\eta = \frac{\pi(p-p')r^4}{8lQ},$$

where η = the viscosity coefficient,
$p-p'$ = the pressure gradient,
r = the radius of the capillary bore,
l = the length of the tube,
Q = the quantity of liquid flowing between two fixed marks in the time t.*

This law has been found to hold with great exactness not only for water, but for most homogeneous solutions. But when Poiseuille, and at a somewhat later date Regnault, examined the rate of flow of blood through a Poiseuille viscosimeter, they met with marked irregularities which they felt bound to ascribe to variations in the composition of the blood.

Except for Donders' reference to Poiseuille's results during the course of a discussion on hæmo-dynamics between Volkmann and Weber, these determinations of the blood viscosity were for many years practically ignored, probably owing to difficulties caused by coagulation. True, in 'Ludwig's Text-Book of Physiology,' published in 1861, we find references to the work of Poiseuille, Darcy, Dubuat, and Girard, and a statement to the effect that "die Geschwindigkeit veränderlich ist mit der Zusammensetzung der Flüssigkeit."

In this country, in 1874, Duncan and Gamgee made some experiments on the rate of flow of blood through tubes of narrow diameter, and observed that the rate of flow of blood taken directly from the vessels of a living animal is very much greater than that of defibrinated blood. This they explained by assuming that in the defibrinated blood there is a tendency for the blood corpuscles to run together and form small corpuscular masses or clumps, which would tend greatly to diminish the rate of flow of the liquid column in which they were suspended, and so might probably give rise to the observed deviations from Poiseuille's law.†

Ewald, working with defibrinated blood in 1877, found a value for its viscosity, which was about five times that of water.‡

A similar result was obtained in 1896 by Nicolls, who used a modified form of Poiseuille's viscosimeter;§ whilst Lewy, also working with defibrinated blood, gave its value as $3\frac{1}{2}$ times that of water.||

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* Poiseuille, 'Ann. d. Chim. et de Phys.,' 3 ser., vol. 1, p. 21, 1847.

† Duncan and Gamgee, 'Journ. Anat. and Physiol.,' vol. 5, p. 184, 1874.

‡ Ewald, 'Arch. f. Physiol.,' Leipzig, 1877.

§ Nicolls, 'Journ. Physiol.,' vol. 20, p. 407, 1896.

|| Lewy, 'Arch. Physiol.,' vol. 70, Bonn, 1897.

In 1894, Graham Brown, writing in the Edinburgh Hospital Reports "On the Changes in the Circulation produced by a Rise in Temperature," pointed out the great diminution in the viscous resistance that occurs when defibrinated blood is made to pass through tubes heated to fever temperature, and applied his results to the explanation of certain phenomena of fever.

Brown also found that if defibrinated blood be rendered lake-coloured by alternately freezing and thawing. the rate of flow was increased, and that at varying temperatures the behaviour of the blood then approached tolerably close to that calculated by the Poiseuille formula.*

Hürthle, alone in 1896, and later with Russell Burton-Opitz, determined the values of the viscosity coefficient of the blood of different living animals by allowing it to flow directly from the carotid through a calibrated tube—simultaneous measurements of the outflow time and of the blood pressure being made. As a result of these experiments Hürthle states that (1) the coefficient of viscosity in any one species of animal is practically constant: that of a dog = 4·5 the value for water at 37° C.; that of a cat = 4·1, and of a rabbit = 3·2; and that (2) almost identically the same values were found for the viscosity of the blood of one and the same individual animal when tubes of various sizes and varying arterial pressures were employed—"from which we are to conclude that the suspension of the corpuscles in the blood does not seriously affect the application of Poiseuille's law to it as a fluid."†

R. Burton-Opitz, using Hürthle's instrument, found that intravenous injections of 0·7 per cent. saline caused an immediate and very distinct decrease in the viscosity, whereas equal amounts of distilled water apparently rendered the blood slightly more viscous. Further, the introduction of alcohol, either directly into the circulation or into the digestive tract, always rendered the blood more viscous: a much greater and more lasting increase appeared when the alcohol was injected into the stomach or duodenum.‡

Hirsch and Beck, employing a modified form of an Ostwald's viscosimeter connected to a constant-pressure apparatus, examined directly the blood of a number of persons: as a mean of their results, they suppose the normal value for the viscosity of human blood to be about five times that of water at 38° C., the lowest value they give being twice, and the highest nine times that of water. By means of this same instrument they examined the blood of 24 patients suffering from nephritis, but were unable to draw any definite conclusions.§

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* Graham Brown, 'Royal Infirmary Reports,' Edin., 1894.

† Professor C. S. Sherrington, on Cardiac Physics, 'Allbutt's Medicine,' vol. 5, p. 476; Hürthle, 'Deutsch. Med. Wochenschr.,' August, 1897.

‡ Burton-Opitz, 'Pflüger's Archiv,' vol. 87, 1900.

§ Hirsch and Beck, 'Deutsch. Arch. für Klin. Med.,' vol. 69, p. 503, 1901, etc.

F. Lommel observed the influence of sweating upon the viscosity of the blood and found, as might have been expected, that in the great majority of cases the viscosity increases owing to the loss of water.*

A. Mayer, in 1901, calculated the coefficients of viscosity of serum, and of normal blood plasma in certain mammals, including man.†

G. Rossi, repeating and extending the observations of Burton-Opitz and A. Mayer, noted the influence of temperature of the viscosity of blood serum. He remarks upon a rather sudden change about the temperature of 45° C., and which is revealed by a marked diminution in the rate at which the viscosity declines and the electrical conductivity is at the same time increased.‡

Fano and Rossi, as a result of their investigations on liquid organic colloids, classify them in two groups according to their behaviour when certain substances as glucose, sodium chloride, etc., are added: in the one group, in which are placed such bodies as gum and starches, the viscosity is considerably diminished by these additions, whereas in the other group, in which are the albumens and various sera, but little effect is produced. However, after subjecting serum to dialysis they found on again adding the above substances that the mixture behaved like a solution of gum or starch; on the other hand, if the dialysed material were added to a solution of a gum, this conducted itself as the original serum.§

Again Fano and Rossi have confirmed Burton-Opitz's observation as to the influence of the thyroid on the blood. Experimenting on dogs and rabbits they found that the removal of the thyroids alone brought about some slight increase in the viscosity; but when the parathyroids also were removed, the viscosity rapidly increased to the time of death, which ensued sooner or later. They assume, therefore, that these bodies produce an internal secretion which is of the nature of an enzyme, whose function is to adjust the chemico-physical conditions of the blood, or, in other words, to regulate the viscosity.||

C. Ferrai drew attention to the marked increase in the viscosity of the blood in asphyxia. He found that it may become double that of arterial blood, and increases in proportion to the increase of CO_2. One element in this increase may be the swelling of the corpuscles under the influence of CO, since the addition of CO_2 to serum, even to saturation, does not increase its viscosity.¶

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* F. Lommel, 'Deutsch. Arch. für Klin. Med.,' vol. 80, p. 830.

† A. Mayer, 'Comp. Rend. de la Soc. de Biol.,' vol. 53, p. 1138, and vol. 54, p. 367, 1904.

‡ G. Rossi, 'Arch. di Fisiol.,' vol. 1, p. 500.

§ Fano and Rossi, 'Arch. di Fisiol.,' vol. 1, p. 609, 1904.

|| Fano and Rossi, 'Arch. di Fisiol.,' vol. 2, 1905.

¶ C. Ferrai, 'Arch. di Fisiol.,' vol. 1, p. 305, 1904.

After we had commenced the experiments described in this paper, we learnt that Dr. R. J. Ewart, of Manchester, had been working at the same subject for some time. At our request he very kindly indicated to us the particular lines of investigation he had pursued. By a very ingenious though complex apparatus he found that the average value for the viscosity of defibrinated pig's blood with a capillary of 0·43 mm. radius is 3·8 times that of water, whereas that of dog's blood is 4·1, cat's, 4·2; whilst that of man is 3·14.

Further, he has carried out experiments on animals asphyxiated with excess of CO and CO_2, and found a rise in the viscosity. He also observed the result of substituting 0·6 per cent. saline for pig's serum, and confirmed the fact that an increase in the number of corpuscles in the blood resulted in an increase in the value of the viscosity coefficient.

Furthermore, he states that the viscosity coefficient of blood flowing through capillary tubes did not vary as the fourth power of the radius, as required by Poiseuille's law.*

Description of Apparatus.

The present experiments were undertaken in the first place to observe :—

(*a*) The influence of the number of corpuscles present upon the viscosity of the blood under varying conditions of temperature and pressure;

(*b*) The effect of the size of different capillary bores upon the rate of flow, again varying the number of corpuscles, temperature and pressure;

(*c*) The alterations, if any, caused by the addition of certain salts and other substances;

and, ultimately,

(*d*) To devise a viscosimeter for clinical purposes which would give reliable results with a very small quantity of blood.

A general idea of the actual arrangement of the various parts of the apparatus employed is most easily obtained from the photographic representation given in fig. 1 (A and B).

In order to study the effect of temperature, four thermostats were maintained at temperatures of approximately 32° C., 36° C., 40° C., and 44° C. respectively. Each thermostat consisted of a large glass beaker, of about 2 litres capacity, containing (*a*) a toluene-mercury gas regulator; (*b*) a thermometer; (*c*) a pear-shaped glass stirrer driven, in series with the other three stirrers, by a small electric motor, shown to the right of the photograph.

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* Ewart, 'Thesis for D.Sc., Liverpool,' 1904.

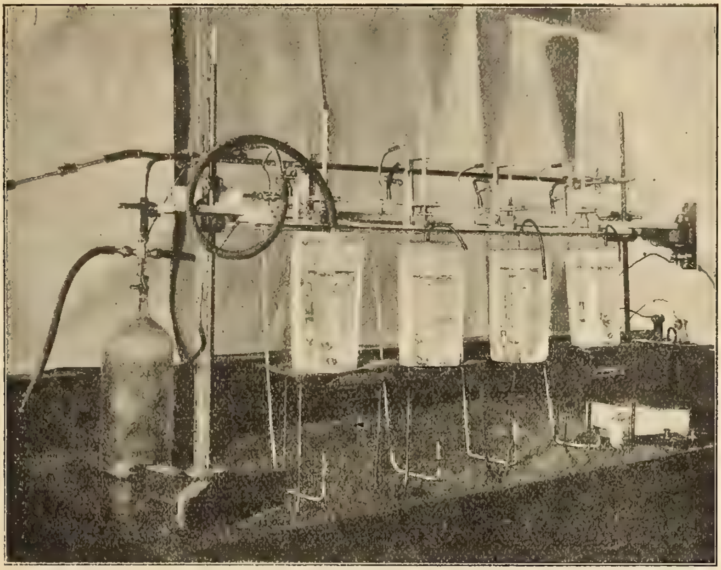

Fig. 1 a.

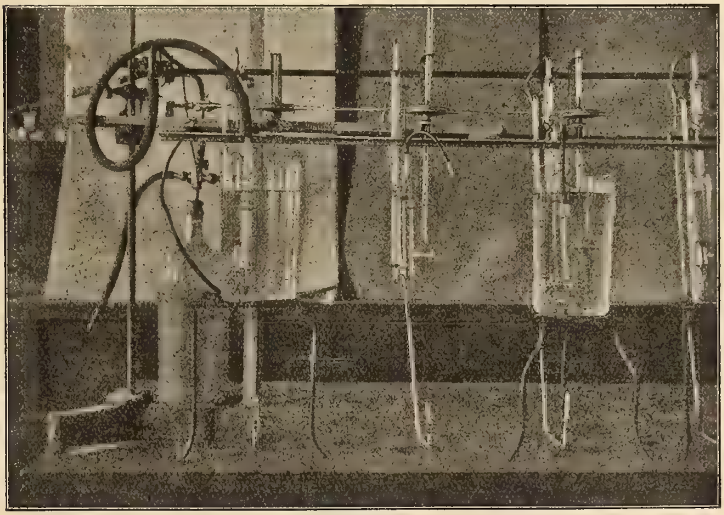

Fig. 1 b.

A diagrammatic representation of the contents of each thermostat, including the viscosimeters placed in position, is afforded by fig. 2. Two wooden bars shown are to be seen placed above the thermostats (fig. 1). To the posterior one were fixed the gas regulators, the thermometers and stirrers, whilst the front bar was notched, and into these notches were fitted the uprights of some special brass clamps, made to hold the viscosimeters vertically.

The kind of viscosimeter used was a slightly modified form of the

U-shaped instrument devised by Ostwald for the comparison of viscosities, and was, in our case, about 20 cm. in length.

A measured quantity of the blood, serum, or other fluid (about 2 c.c. with the smaller tubes) was introduced by means of a calibrated pipette down the wider arm into the larger bulb near the bend of the U. As will be seen from the diagram (fig. 3), the other limb of the viscosimeter was made of capillary tubing with a second and smaller bulb blown about 14 cm. above the bottom bend. The capillary tube above this smaller bulb was generally of wider bore and bent some four or five times away from the vertical, alternatively right and left as shown; in order to still further reduce as much as possible any irregularities or disturbances resulting from a too rapid sedimentation of the corpuscles at the bend joining the two limbs of the viscosimeter, the bend consisted of tubing of enlarged bore whilst the blood itself was well mixed by previously bubbling a slow stream of air through it before measuring the rate of flow.

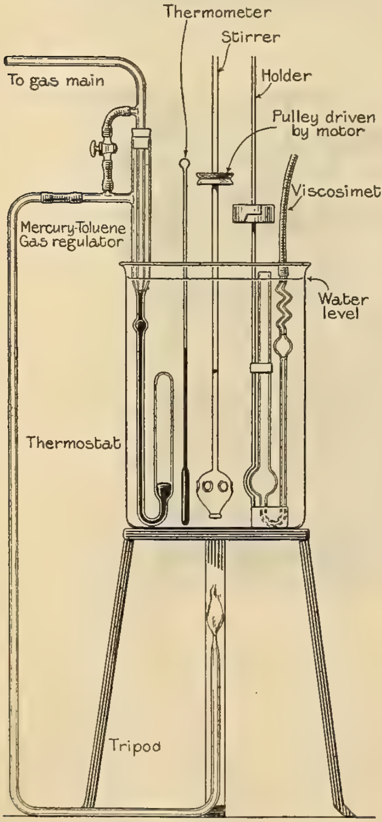
Thermometer
Stirrer
Holder
To gas main
Pulley driven by motor
Viscosimeter
Mercury-Toluene Gas regulator
Water level
Thermostat
Tripod

FIG. 2.—Diagram to show Arrangement of Apparatus in Thermostat.

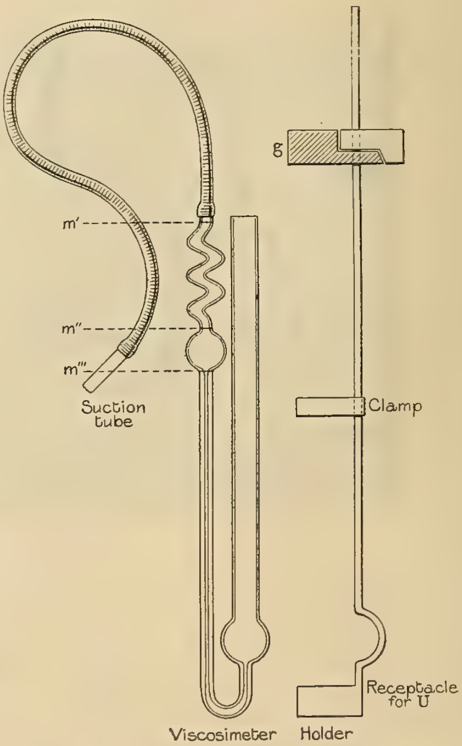
g
m′
m″
m‴
Suction tube
Clamp
Receptacle for U
Viscosimeter
Holder

FIG. 3.—Diagram of Viscosimeter and Holder.

After sufficient time had been allowed to elapse for the viscosimeter and its contents to acquire the temperature of the bath in which it was placed, the blood or other fluid content was sucked up to the point marked (m') by means of a piece of rubber tubing affixed to the capillary arm, and then permitted to flow back through the bulb under the action of gravity. By means of a stop watch, reading to one-fifth of a second, the time taken for the end of the fluid column to flow from m'' to m''' was observed. By thus allowing the liquid column to get up a steady motion before observing its rate of flow we eliminate to a great extent errors resulting from differences of inertia of the various liquids examined.

If t' is the time in seconds for water at any particular temperature, σ' its specific gravity, whilst t is the time for the fluid examined and σ its specific gravity, then the relation between their viscosity coefficients η' and η respectively is

$$\eta : \eta' = \sigma t : \sigma' t', \quad \text{or} \quad \eta = \eta' \frac{\sigma t}{\sigma' t'}. \qquad \text{Formula (II)}$$

The absolute value taken for the viscosity coefficient of water, that is for η', at 35° was 0·007361 C.G.S. units, the value given by Thorpe and Rodger.

For those experiments in which the pressure gradient was varied the narrower limb of the viscosimeter was connected to a large Winchester bottle containing compressed air, by means of a long piece of pressure tubing wired on to the brass tube F.E. (*vide* fig. 4). Through the rubber bung in the neck of the Winchester was passed a +-shaped brass union which had taps on three of its arms; one of these, C, was in communication with a force pump, another, B, with a mercury manometer; and the third, A, was connected to the brass tube leading to the viscosimeter. A second brass tube, leading to an exhaust (filter) pump and provided with a tap at D, was soldered perpendicularly into F.E.

Before using this accessory apparatus the tap A was closed, air was forced into the Winchester until the manometer registered a convenient height, and the tap C then closed. The liquid in the viscosimeter was now drawn up to the topmost mark (m') by opening the tap D, and thereby connecting to the filter pump. When D was closed, A was opened, and the one observer with the stop-watch now noted the time of flow between the middle and lowest marks on the viscosimeter tube as before, whilst the other noted the initial and final pressure readings and manipulated the taps.

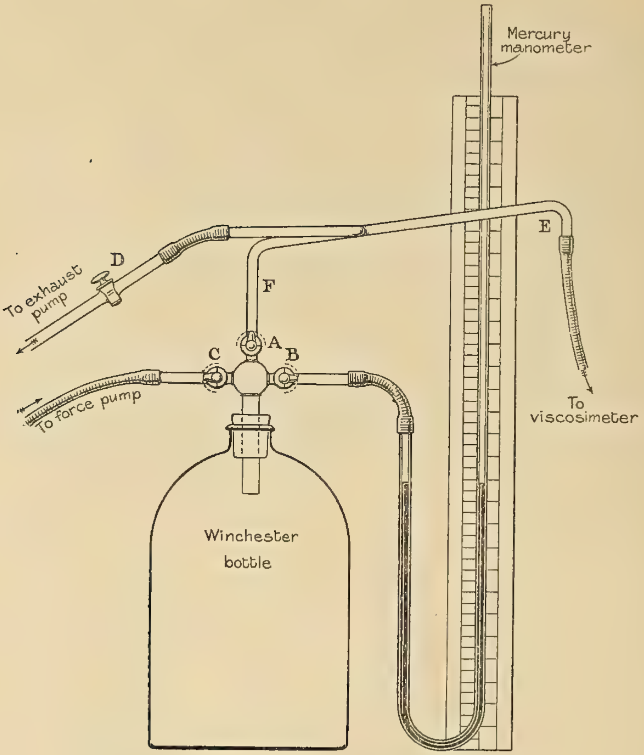

FIG. 4.—Accessory Pressure Apparatus.

Details of Experimental Results.

For the immediate purpose of these laboratory experiments, horses' blood was employed, since the corpuscular elements in such blood have been estimated by Sir John McFadyean to be practically identical in shape, though slightly less (in size) than those of human blood, viz., about 7 μ in diameter, and, moreover, horses' blood has this distinct advantage that, after an anti-coagulator has been added to it, the corpuscles and other cellular bodies are comparatively quick in settling, and soon leave a reasonably clear plasma. The objection may, perhaps, be raised that experiments carried out with animal blood are not, strictly speaking, applicable to human beings, but, as will be shown in the sequel, such results, although not numerically applicable, cannot but inevitably lead to deductions and conclusions, the principles of which are as fundamentally true for human physiology as for animal physiology.

Considering the heterogeneous structure of blood in general, and how variously the constituents of the blood of individual members of any one species may differ among themselves, evidently if one desire to obtain strictly comparable results under varying conditions, say of the effect of corpuscles present, or of the effect of adding different quantities of the same or different salts, and to draw trustworthy deductions from the results of such experiments, it is absolutely essential that these experiments should, as far as possible, be carried out with blood obtained from the same animal or human specimen at one and the same time, and from the same part of the body, whilst the experimental conditions should be such as to allow the greatest amount of control of the particular influence it is desired to investigate. Clearly a vein or artery, probably in an unsymmetrical position with variable walls and connected directly to a complicated circulatory system with possible fluctuations of pressure, is not the ideal place to study in the first instance, say the effect of varying the number of corpuscles or that of different quantities of any particular salt.

The method we habitually adopted, after drawing up our scheme of experiments, was to place into a number of wide-mouthed stoppered jars just so much solution of a calculated strength of the anticoagulator chosen that, in general, when the stoppered jar was brought under the open vein of the animal and allowed to fill up to a definite mark, we knew that for every 95 c.c. of blood we had 5 c.c. of water and a known amount of the anticoagulator—or 5 c.c. of the anticoagulator when the same was not a water solution, *e.g.*, oil. (In the case of additions of $MgSO_4$, more water was present.) The jars thus filled were allowed to stand for two or three hours to settle. A large quantity of the supernatant plasma was then drawn off and centrifuged for two minutes.

To successive portions of this clear centrifuged plasma systematic additions of red cells were made, and a careful blood count taken from a well-shaken portion of this artificial blood by means of a Thoma-Zeiss hæmocytometer, just before the required amount was placed in the viscosimeters.

Of the fairly representative group of anticoagulators we tried—viz., potassium oxalate, sodium citrate, magnesium sulphate, peptone, leech extract, and olive oil—the two first proved to be the most satisfactory for our work. In Tables I and II will be found three series of observations made with different concentrations of each of these two anticoagulators at different temperatures and with varying numbers of red corpuscles suspended in similar plasma.

Under η in each sub-section are tabulated in absolute units the values found for the viscosity coefficient at the temperatures recorded in the first column on the left, with a blood containing the number of corpuscles per cubic millimetre stated at the head of the column. Under R is given the ratio of this value of η for blood to that of water at 35° C. (viz., 0·007361 C.G.S. units).

The values given for the specific gravity are those used in making the necessary calculations of η by means of Formula II. Most of these values were obtained experimentally: from the data thus supplied the others have been obtained by interpolation on the assumption based on Hayem's statement that the specific gravity depends on the corpuscular richness of the blood, although it may be remembered that Schmaltz, judging from observations with his capillary pycnometer, concludes that the percentage of hæmoglobin, and not the number of corpuscles, is the main determining factor.

Table I.—Showing the Effect on the Viscosity Coefficient of varying (*a*) the number of red corpuscles, (*b*) the amount of salt added.

Viscosimeter bore = 0·6 mm. in diameter.

(A) Anticoagulator—5 c.c. of 0·1 per cent. potassium oxalate per 100 c.c.

No. of red corpuscles per cub. millim. ... Specific gravity	0 1·030		1·6 × 10^6 1·034		4·0 × 10^6 1·042		8·4 × 10^6 1·055	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0144	2·0	0·0200	2·7	0·0274	3·7	0·0637	8·7
35·0	0·0137	1·9	0·0186	2·5	0·0258	3·5	0·0554	7·5
40·0	0·0120	1·6	0·0165	2·3	0·0234	3·2	0·0456	6·2
44·8	0·0108	1·5	0·0153	2·1	0·0214	2·9	0·0376	5·1
Mean temp. coeff. ...	0·00028	—	0·00036	—	0·00046	—	0·00201	

(B) Anticoagulator—5 c.c. of 0·2 per cent. potassium oxalate per 100 c.c.

No. of red corpuscles per cub. millim. ... Specific gravity	0 1·030		1·5 × 10^6 1·033		4·2 × 10^6 1·043		6·2 × 10^6 1·050	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0137	1·9	0·0176	2·4	0·0254	3·5	0·0280	3·8
35·0	0·0128	1·7	0·0160	2·2	0·0231	3·2	0·0259	3·5
40·0	0·0118	1·6	0·0139	1·9	0·0205	2·8	0·0229	3·1
44·8	0·0106	1·4	0·0128	1·7	0·0182	2·5	0·0209	2·8
Mean temp. coeff. ...	0·00024	—	0·00037	—	0·00055	—	0·00055	

(C) Anticoagulator—5 c.c. of 0·3 per cent. potassium oxalate per 100 c.c.

No. of red corpuscles per cub. millim. ... Specific gravity	0 1·030		$1\cdot4 \times 10^6$ 1·033		$4\cdot2 \times 10^6$ 1·043		$8\cdot4 \times 10^6$ 1·055	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0134	1·8	0·0167	2·3	0·0184	2·5	0·0287	3·9
35·0	0·0125	1·7	0·0157	2·1	0·0176	2·4	0·0272	3·7
40·0	0·0115	1·6	0·0140	1·9	0·0159	2·2	0·0257	3·5
44·8	0·0104	1·4	0·0126	1·7	0·0143	1·9	0·0236	3·1
Mean temp. coeff. ...	0·00024	—	0·00032	—	0·00032	—	0·00039	

Table II.

(A) Anticoagulator—5 c.c. of 0·5 per cent. sodium citrate per 100 c.c.

No. of red corpuscles per cub. millim. ... Specific gravity	0 1·030		$2\cdot6 \times 10^6$ 1·037		$4\cdot0 \times 10^6$ 1·042		$8\cdot0 \times 10^6$ 1·054	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0140	1·9	0·0242	3·3	0·0296	4·0	0·0846	11·1
35·0	0·0128	1·7	0·0222	3·0	0·0268	3·7	0·0708	9.6
40·0	0·0114	1·6	0·0194	2·6	0·0233	3·2	0·0562	7·6
44·8	0·0103	1·4	0·0163	2·2	0·0198	2·7	0·0433	5·8
Mean temp. coeff. ...	0·00028	—	0·00061	—	0·00076	—	0·00317	

(B) Anticoagulator—5 c.c. of 1 per cent. sodium citrate per 100 c.c.

No. of red corpuscles per cub. millim. ... Specific gravity	0 1·030		$2\cdot1 \times 10^6$ 1·035		$4\cdot4 \times 10^6$ (?) 1·043		$5\cdot8 \times 10^6$ 1·049	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0142	1·9	0·0203	2·8	0·0264	3·6	0·0324	4·4
35·0	0·0132	1·8	0·0190	2·6	0·0244	3·3	0·0299	4·1
40·0	0·0118	1·6	0·0168	2·3	0·0221	3·0	0·0264	3·6
44·8	0·0106	1·4	0·0149	2·0	0·0196	2·7	0·0235	3·2
Mean temp. coeff. ...	0·00028	—	0·00042	—	0·00052	—	0·00062	

(C) Anticoagulator—5 c.c. of 2·5 per cent. sodium citrate per 100 c.c.

No. of red corpuscles per cub. millim. …	0		$2·7 \times 10^6$		$5·4 \times 10^6$		$9·2 \times 10^6$	
Specific gravity ……	1·030		1·037		1·048		1·052	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
31·8	0·0138	1·9	0·0188	2·6	0·0253	3·5	0·0319	4·3
35·0	0·0128	1·7	0·0175	2·4	0·0234	3·2	0·0292	4·0
40·0	0·0115	1·6	0·0160	2·2	0·0207	2·8	0·0257	3·5
44·8	0·0109	1·5	0·0146	2·0	0·0185	2·5	0·0228	3·1
Mean temp. coeff. …	0·00021	—	0·00032	—	0·00052	—	0·00072	

Table III.

(A) Anticoagulator—5 c.c. of 0·3 per cent. potassium oxalate per 100 c.c.

No. of red corpuscles per cub. millim. …	0		$3·2 \times 10^6$		$6·3 \times 10^6$		$12·6 \times 10^6$	
Specific gravity ……	1·042		1·049		1·059		1·078	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
32·2	0·0139	1·9	0·0244	3·3	0·0356	4·9	0·1150	15·6
35·0	0·0131	1·8	0·0226	3·1	0·0340	4·6	0·1030	14·0
40·4	0·0122	1·7	0·0195	2·7	0·0309	4·2	0·0820	11·2
44·8	0·0111	1·5	0·0171	2·3	0·0285	3·9	0·0706	9·6
Mean temp. coeff. …	0·00022	—	0·00058	—	0·00057	—	0·00352	

(B) Anticoagulator—5 c.c. of 0·1 per cent. potassium oxalate per 100 c.c.

No. of red corpuscles per cub. millim. …	0		$2·0 \times 10^6$		$4·0 \times 10^6$		$8·8 \times 10^6$	
Specific gravity ……	1·042		1·045		1·055		1·067	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
32·2	0·0141	1·9	0·0206	2·8	0·0330	4·5	0·0602	8·2
35·0	0·0134	1·8	0·0191	2·7	0·0313	4·3	0·0575	7·8
40·4	0·0120	1·6	0·0165	2·3	0·0282	3·8	0·0530	7·2
44·8	0·0110	1·5	0·0149	2·0	0·0264	3·6	0·0481	6·5
Mean temp. coeff. …	0·00025	—	0·00045	—	0·00052	—	0·00096	

(C) Anticoagulator—5 c.c. of 0·5 per cent. sodium citrate per 100 c.c.

No. of red corpuscles per cub. millim. … Specific gravity ……	0 1·042		2·7 × 10^6 1·047		5·4 × 10^6 1·055		10·8 × 10^6 1·072	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
° C.								
32·2	0·0143	1·9	0·0254	3·5	0·0375	5·1	0·1225	16·9
35·0	0·0135	1·8	0·0234	3·2	0·0350	4·8	0·1090	14·8
40·4	0·0123	1·7	0·0196	2·7	0·0306	4·2	0·0880	12·1
45·0	0·0111	1·5	0·0158	2·1	0·0268	3·7	0·0690	9·5
Mean temp. coeff. …	0·00024	—	0·00075	—	0·00083	—	0·00417	

However, these specific gravity values are not of great importance, since the degree of accuracy obtainable with a Thoma-Zeiss or any form of hæmocytometer limits the degree of accuracy of the other measurements.

The Effect of Varying the Number of Corpuscles.—A most casual glance at either of these tables or at Table III will at once show that the increase of corpuscles always caused a marked increase in the values of η, and may under some circumstances result in a disproportionately large increase, the values in Table I ranging from about 1·5 times that of water at 35° C. to as much as 8·7 times (Sub-section A), or in Table III to 15·6 times the value for water (Sub-section A).

This result is, however, most simply brought out by the curves in fig. 5, in which are plotted a few values obtained at 35° C. (taken from Table I), with the number of corpuscles as ordinates and the value for η as abscissæ.

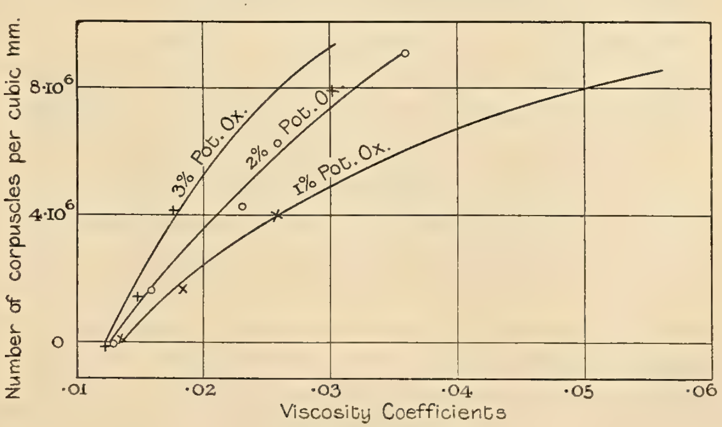

FIG. 5.—Curves showing Effect of Corpuscles on Viscosity with different amounts of Potassium Oxalate present. Temp. = 35° C. (*Cf.* Table I.)

Except for the different percentages of added anticoagulator, the blood was the same in each case.

Inasmuch as the curves are not straight lines, it is evident that the viscosity is not directly proportional to the increase of corpuscles, but rather that the former may increase very much more rapidly than the latter—obviously in the present case more so when 0·1 per cent. potassium oxalate was added than when it was 0·3 per cent. potassium oxalate. Similar curves, although not of the same curvature, were obtained when other experimental values were plotted. There are diverse reasons for the dissimilarity, as will be subsequently evident. In fig. 6, on precisely the same scale, are recorded results obtained with a different blood, namely, that of a young horse (3 years old), whilst the former was that of an old one (about 18 years old). But, though the same strengths of anticoagulators were again added, it is apparent that the increase in viscosity was here much greater for any given increase in the number of corpuscles. Indeed, from the trend of these three curves it may well be supposed that a blood containing upwards of 20×10^6 corpuscles per cubic millimetre would have taken longer to flow through the particular viscosimeter tubes used than the same quantity of treacle would have done. It may further be observed that the differences of the influence exerted by each of the three anticoagulators are not so strongly marked in this set of curves.

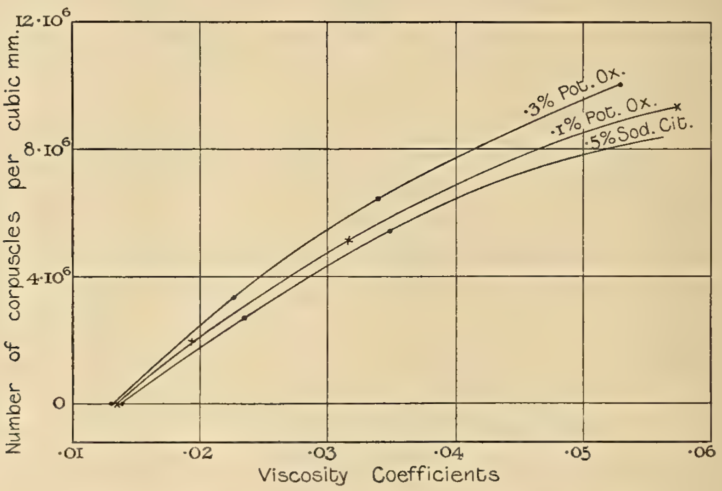

Fig. 6.—Curves plotted from Table III. Temp. = 35°.

Such results as the foregoing are obviously in direct contradiction to Hürthle's view which Professor Sherrington quotes in Allbutt's 'Medicine,' and to which reference has already been made, viz., that "the suspension of the corpuscles in the blood does not seriously affect the application of Poiseuille's law to it as a fluid."

From the results of some of his experiments, Dr. Ewart has arrived at the rather astonishing result that a blood containing $5 \cdot 10^6$ corpuscles per cubic millimetre requires least expenditure of energy on the part of the heart to drive a definite quantity of it in unit time past any particular cross-section, or, as he says, human blood containing five million corpuscles per cubic millimetre is mechanically the most efficient and economical. Similar calculations from our observations, however, have not afforded the slightest confirmation of such an unexpected result. Nor do Dr. Ewart's own results always agree with it.

It would, moreover, appear to us that the deduction is by no means justifiable or, indeed, valid, inasmuch as the calculations rest upon direct comparison between three different kinds of blood, viz., pig's blood for the value with the $6 \cdot 10^6$ corpuscles, human blood for that with $5 \cdot 10^6$, and a mixture of human and pig's serum for the lower values. As will be seen later, the mere number of corpuscles present is far from being the only factor which may influence the viscosity of a blood.

The Effect of Change of Temperature.—At the bottom of each sub-section is given the change in viscosity which resulted from a change of temperature of 1° C., that is, the mean temperature coefficient at temperatures of about blood heat. And if we take any one series, it is apparent that this temperature coefficient increased with increase in the number of corpuscles. In figs. 7 and 8 are plotted the values of the viscosity coefficient found at different temperatures for the three series, Table II (B), and Table III (A)

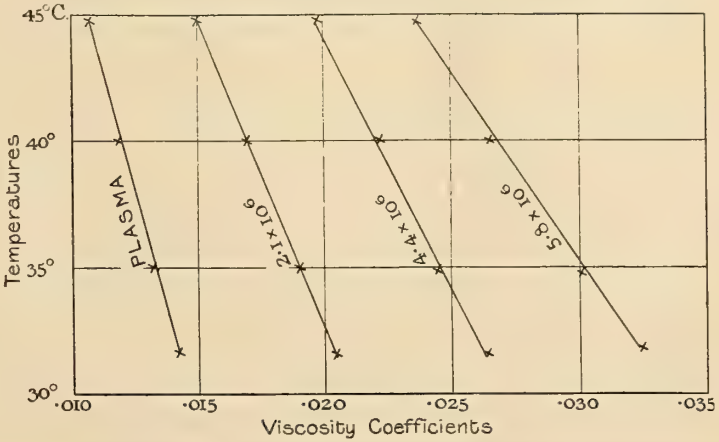

FIG. 7.—Curves showing Temperature Effect with varying number of Corpuscles. (*Cf.* Table II [B].)

and (C) respectively—the number of corpuscles being given by the side of each curve. The differences in slope obviously form a graphical confirmation of the foregoing remarks with regard to the temperature coefficient, whilst the numerical details supplied in the tables show that it ranged from about 0·0002 to 0·004. Although the temperature gradients are represented by straight lines in these diagrams, experiments over a larger range showed that the change of viscosity with temperature was by no means a linear function.

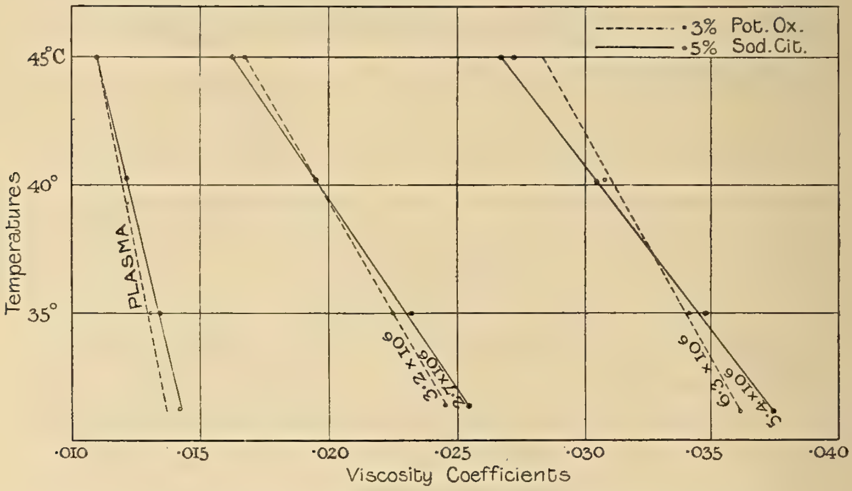

Fig. 8.—Curves contrasting Temperature Variations after Additions of Potassium Oxalate and Sodium Citrate solutions respectively. (*Cf.* Table III [A] and [C].)

The Effect of Added Salts, etc.—The possible influence which the addition of salts may have upon the relation of the viscosity to the number of corpuscles will have already been gathered both from the tables of numerical results and from the curves, of which mention has so far been made. From fig. 5 it will appear that the addition of 0·3 per cent. potassium oxalate reduced the viscosity coefficient of blood containing $8{\cdot}4 \times 10^6$ corpuscles per cubic millimetre to less than half of that with blood containing 0·1 per cent. potassium oxalate, but otherwise similar in composition; whereas from fig. 6 the difference caused by the added salts is hardly noticeable with the three anti-coagulators mentioned in the figure. Again, fig. 8 would apparently indicate that 0·3 per cent. potassium oxalate could evidently reduce the viscosity more than 0·5 per cent. sodium citrate, whilst the mean temperature coefficient would seem to be larger for the sodium citrate solution, inasmuch as the potassium oxalate temperature curves are steeper.

Although it is evident in the cases just considered that the addition of these salts decreased the viscosity, yet the extent of the reduction is apparently quite a variable quantity; indeed it depends to a large extent upon the internal composition of the particular blood examined, and on the initial action of its constituents and the added salts.

Ewart has also observed that additions of dilute saline lowered the viscosity, whereas an increase of acidity or alkalinity, as well as the addition of many neutral salts, caused an increase in viscosity. The fall in viscosity he attributes to a reduction of the quantity of albumin, etc., and the fall of proteid strength.

When considering the effects of changing the concentrations of the salt solutions, etc., present, it should be borne in mind that whenever an animal cell is brought into a strong salt solution, the osmotic pressure of which is greater than that of the cell sap, the cell contracts and becomes flaccid, owing to the passage outwards of certain constituents of the sap, whilst the effect of placing the cell into a dilute solution, the osmotic pressure of which is less than that of the cell sap, will cause the cell to expand and even eventually burst. As hæmatocrite and other experiments have shown, the blood corpuscles are affected in the same way; in strong solutions they are crenated; in dilute solutions they may burst and allow the red colouring matter to pass into the surrounding liquid. Duncan and Gamgee have shown that laking causes a diminution of viscosity.

But the study of the effect of changing the concentration of the chemical bodies present in the blood is still further complicated by the fact that the protoplasmic cell walls may vary considerably in their permeability. For instance, Dr. Loeb, of Chicago, asserts that the eggs of sea urchins, if placed in concentrated solutions of NaCl, die at once, but show increased vitality if a tiny quantity of certain metallic salts be present. Experimenters with colloidal liquids have frequently noticed how the addition of certain reagents may sometimes bring about changes in some particular property of the colloid, which are altogether out of proportion to the weight or bulk of the added reagent, *e.g.*, one drop of a weak $FeCl_3$ solution to about 50 c.c. of colloidal $Fe(OH)_3$ has been observed to lower the viscosity of the latter by 500 per cent.* Whilst Majorana† and Schmaus‡ have found that the smallest traces of the chloride were sufficient to destroy all traces of the magnetic double refraction of the colloidal iron hydrates, etc.

The study of the influence of various salts upon the viscosity of the blood is obviously a province in which much work has yet to be done in order to find out which chemical bodies most strongly affect the viscosity of the blood under certain conditions, since this is clearly of importance in considering changes in the circulatory system. Mention may be made of the recent researches of G. Stodel on the biological importance of small quantities of colloidal bodies in the treatment of certain infective diseases, etc. An investigation of the effects of these bodies on the viscosity of the blood may conceivably prove most profitable.

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* *Vide* Denning, 'Ueber die Viscosität, etc., des colloidalen Eisenoxydhydrates, Inaugural-Dissertation, Heidelberg, 1904.

† Qu. Majorana, 'Rendic. Acc. del Lincei,' II, vol. 1, p. 374, etc., 1902.

‡ Schmaus, 'Ann. der Physik,' vol. 10, p. 658, vol. 12, p. 186, 1903.

A subsequent series of determinations some six days later gave practically the same results.

The Viscosity Coefficients for Various Plasmata and Sera obtained from the blood of the same animal are placed together in Table IV, with the anticoagulator into which the blood was received at the head of each column.

Table IV.—Showing Values of the Viscosity Coefficients found for various Plasmata and Sera.

Anticoagulator.	Salted plasmata.		Sera.		
	0·3 per cent. potassium oxalate.	12·5 per cent. $MgSO_4$.	Peptone.	Leech extract.	Olive oil.
° C.					
31·8	0·0139	0·0141	0·0136	0·0126	0·0125
35·0	0·0131	0·0134	0·0128	0·0119	0·0119
40·0	0·0122	0·0124	0·0116	0·0104	0·0105
44·5	0·0110	0·0115	0·0105	0·0098	0·0099

With horses' blood, as is well known, there follows a rapid subsidence of the corpuscles: consequently it was easy to siphon off small quantities of the supernatant liquid from each specimen. In the case of the first two specimens (namely, with potassium oxalate and $MgSO_4$) observations were taken before coagulation had occurred, *i.e.*, we were dealing with plasma; whilst in the case of the last three clotting had occurred before estimations were attempted. Having found such small variations in the viscosity values for the plasma and serum with the conditions under which these experiments were made, we did not pursue this question further, inasmuch as our chief object was to investigate those factors which play a predominant part in determining the viscosity of the blood.

The Effect of Varying the Capillary Bore.—When it is remembered that blood is really a very complex colloidal suspension or emulsion, containing numerous particles, corpuscles, and other cellular elements, in a feebly viscous matrix, we may well imagine that the rate of increase of the viscosity coefficient with each fresh addition of corpuscles, etc., will depend largely on the size of the capillary bore of the viscosimeter, and with different bloods on the relative sizes of the corpuscular elements. Naturally we cannot expect the addition of the corpuscles *per se* as semi-solids, and their variations in size, to have any appreciable effect on the viscosity unless they are to pass through capillary tubes of radius comparable with their own dimensions. Against Hürthle's statement, already mentioned, that "almost identically the same values were found for the viscosity of the blood of one and the same individual animal when tubes of various sizes and varying arterial pressures were employed," it is to be urged that his method of attacking the question did not admit of his obtaining a sufficient experimental range from which reliable conclusions could be drawn. From the experimental results recorded in Table V, for which a large quantity of blood was available, whilst viscosimeter tubes of bores varying from 2 mm. to 0·3 mm. could be directly employed, and "artificial" blood mixtures with widely differing numbers of corpuscles easily made up, it will be seen that the smaller influences of each successive alterations become, as it were, of magnified importance, when viewed as parts of a bigger scheme, and show at once that blood containing a large number of corpuscles encounters considerably greater resistance to its flow than is to be expected from an application of Poiseuille's law, in its

Table V.—Showing the Variation of the Viscosity Values with the size of the Capillary Bore.

Capillary bore.	2 mm.		1 mm.		1·6 mm.		0·3 mm.	
Temperature.	η.	R.	η.	R.	η.	R.	η.	R.
Plasma from 1 per cent. potassium oxalate.								
32°·2	0·0130	1·8	0·0139	1·9	0·0140	1·9	0·0141	1·9
40·4	0·0110	1·5	0·0119	1·6	0·0121	1·6	0·0121	1·6
Plasma + 3·6 × 10^6 corpuscles per cubic millimetre.								
32·2	0·0202	2·7	0·0241	3·3	0·0272	3·7	0·0338	4·6
40·4	0·0180	2·4	0·0221	3·0	0·0244	3·3	0·0304	4·1
Plasma + 6·0 × 10^6 corpuscles per cubic millimetre.								
32·0	0·0265	3·6	0·0333	4·5	0·0402	5·5	0·0546	7·4
40·4	—	—	0·0302	4·1	0·0368	5·0		
Plasma + 9·6 × 10^6 corpuscles per cubic millimetre.								
32·0	0·0474	6·5	0·0562	7·5	—	—	0·0982	13·4
40·4	0·0428	5·8	0·0513	7·0				

passage through fine capillaries than it does through tubes of comparatively wide bore.

During the consideration of the series of observations contained in Table III we drew attention to the diversity of the results that might be obtained with the blood of different animals, even though it contain exactly the same number of corpuscles and the same amount of added salt or anticoagulator. This at first sight may seem very contradictory; but remembering how the red cells vary in size not only in different species but in the individuals of the same species, we would suggest, from considerations of physical and natural phenomena, that this dissimilarity may be due to quite small variations in the dimensions of the red cells and the amount of the attendant colloidal matter (*e.g.*, proteids, etc.) enveloping them as central nuclei in much the same way as, for example, the solid and semi-solid particles in brackish water are coated with slime, or as the planets surrounded by their attendant atmospheres, or perhaps more nearly resembling the richly colloidal *Schaummassen* and *Schaumflocken* in Professor Quincke's theory of colloidal solution.*

And, indeed, here it might well be remarked that just as all purely colloidal solutions which have been standing for any length of time tend to become more viscous by reason of the growth of existing colloidal nuclei and the formation of new ones at the expense of the colloidal materials in the sustaining fluid medium, so we may similarly expect that a badly circulating but otherwise healthy blood with the average amount of colloids would, as a result of its partial stagnation, likewise tend to become more viscous, and so, by thus still further decreasing the circulation through the whole system, and more especially in the peripheral vessels, eventually give rise to some form of cyanosis, unless counteracted in other ways.

As showing the comparative unimportance of such changes to the rate of flow through the veins and other large vessels, we give, in Table VI, a few comparative values obtained with a tube of 3·5 mm. and a capillary of 0·6 mm. It has, of course, long been recognised that Poiseuille's law only holds for homogeneous solutions in narrow tubes if the rate of flow be slow, which certainly was not the case with the larger tube in question, as may, perhaps, be inferred from the low value found for the plasma. But for our purpose this is immaterial, since the result clearly illustrates the fact that changes in the viscosity are only of importance in the circulation of the blood through the finer channels of the circulatory system. For example, it will be seen that, with a blood containing some 16 million red corpuscles per cubic millimetre, the value for η was only 5·3 times that for water at

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* *Cf.*, *e.g.*, G. Quincke, 'Ann. d. Phys.,' vol. 9, pp. 969—1045, 1902.

Table VI.—Giving Comparative Values between a Wide Tube and a Capillary.

Bore.	3·5 mm.				0·6 mm.	
Temperature.	Plasma.		Plasma + $16 \cdot 10^6$ corpuscles per cub. millim.			
	η.	R.	η.	R.	η.	R.
° C.						
32·2	0·0091	1·24	0·0386	5·3	0·208	28·4
45·0	0·0086	1·17	0·0288	3·9		
18·2	—	—	0·0745	10·2	0·541	74·0

35° C. with the big tube, but 28·4 with the smaller, whilst determinations made at the atmospheric temperature, viz., 18°·2 C., gave values of 10·2 and 74 respectively, showing the absolute futility of taking measurements of viscous blood without simultaneously recording the temperature and the approximate bore of the narrow portion of the viscosimeter, for example, as Dr. F. Parkes Weber* has neglected to do.

The Effect of Change of Pressure.—The results of a series of experiments carried out with the accessory apparatus, shown in fig. 4, are given in Table VII. The pressure range adopted lay between 17·6 cm. Hg and 2·2 cm., or was somewhat larger than that generally met with in the human system. The corrected values for the pressures, that is to say, the readings of the manometer and a correction for the difference of level of the blood in the viscosimeter tubes, are tabulated in the first column of each sub-section, the outflow time in seconds in the second column, and the calculated values of η in absolute units in the third. Obviously if the different specimens of blood obeyed Poiseuille's law for these changes of pressure the product "pressure × time" should be a constant, that is, the calculated values for η should be constant. For the result recorded in the first two sub-sections this will be seen to hold within the limits of experimental error.

But for the blood containing $6 \cdot 10^6$ corpuscles per cubic millimetre, and more especially for that $9 \cdot 6 \times 10^6$ in a tube of 3 mm. diameter, it is evident that a gradual decrease of pressure resulted in a gradual increase in the η. Or with a fine capillary (*e.g.*, animal capillaries) the time of outflow is not simply inversely proportional to the pressure as required by the Poiseuille formula. With tubes of wider bore, and over the same range of pressure and number of corpuscles, such marked deviations were not detectable.

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* 'Clinical Society's Transactions,' 1904.

Table VII.—Showing Values of η under Different Pressures with varying Number of Corpuscles.

Capillary bore = 0·3 mm. Temperature = 32°·2 C.

Pressure (corr.).	Time of outflow.	η.	Pressure (corr.).	Time of outflow.	η.
Plasma.			+ 3·6 × 10^6 corpuscles per cubic millimetre.		
Cm. Hg.	Secs.	C.G.S. units.	Cm. Hg.	Secs.	C.G.S. units.
15·6	22·8	0·0138	12·8	65·8	0·0332
13·5	27·4	0·0143	10·1	85·0	0·0338
11·3	33·4	0·0145	7·7	112	0·0342
8·0	47·2	0·0145	5·0	168	0·0332
4·6	80·3	0·0142	2·2	410	0·0347
2·6	141·7	0·0139			
+ 6 × 10^6 corpuscles per cubic millimetre.			+ 9·6 × 10^6 corpuscles per cubic millimetre.		
16·0	85·0	0·0538	17·6	138·0	0·0964
14·4	95·2	0·0550	15·6	158·0	0·0972
10·4	134·0	0·0552	10·8	229·4	0·0984
7·3	188·0	0·0545	8·2	306·0	0·0998
—	—	—	5·6	306·0	0·1079

With very fine tubes and long times of outflow it may be mentioned that the segregation of corpuscles at the bottom of the viscosimeter is liable to cause annoying irregularities, involving frequent repetition after the blood has been well mixed by gently bubbling a slow stream of air through it. From the curves of fig. 9 which, it will be noticed, are practically rectangular hyperbolas of decreasing curvature, some interesting inferences may be drawn. The outflow times of fig. 9 have been plotted as abscissæ and the

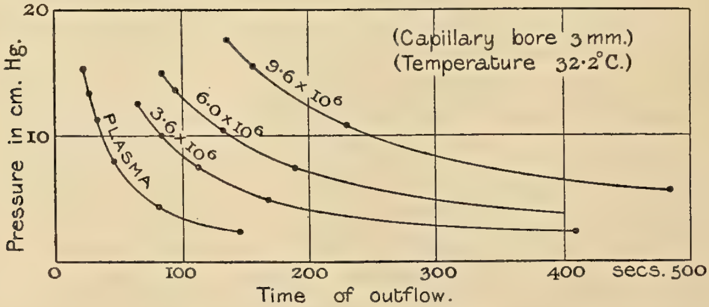

Fig. 9.—Curves showing the Relation between Outflow Times and Pressures with varying amounts of Corpuscles.

pressures as ordinates. Over the present range of pressures it will be seen that a small change of pressure at, *e.g.*, 10 cm. Hg, will cause a greater diminution of the outflow time for blood poor in corpuscles than for blood rich in corpuscles and, as a consequence of this, in the human circulatory system we should find such changes producing a much more rapid return of the arterial blood to the heart. Further reference will be made to this point, however, in the subsequent discussion of the bearing of this and foregoing results on the disorders of the circulatory system. Below about 5 cm. pressure the curves would seem to indicate that this effect is not so much *en evidence.* Similar results and curves, though naturally with smaller values for η, were obtained when these observations were repeated at higher temperatures.

Summary.

The chief results of the foregoing investigations may be briefly summarised in the following general statements:—

1. The decrease in viscosity for each degree rise of temperature is less marked for plasma than for blood.
2. It is also decidedly less for blood containing few corpuscles per cubic millimetre than for blood containing many corpuscles per cubic millimetre.
3. For any given temperature and capillary bore an increase in the number of corpuscles causes an increase in the viscosity (*cf.* Tables I–III), though it is to be remarked that—
   (*a*) with tubes of wide bore a very large increase in the number of corpuscles is necessary to cause an appreciable effect (*cf.* Table VI), whereas
   (*b*) with small capillaries a slight increase in the number of corpuscles always causes a very marked increase in the viscosity (*cf.* Table V).
4. With a given number of corpuscles the rate of flow through any particular tube down to about 3 mm. in diameter is, over the range of pressure occurring in living organisms, practically directly proportional to the pressure.
5. A given increase of pressure exerts a much greater accelerating effect on the rate of flow through tubes of fine calibre than through tubes of wider bore.
6. The influence of a definite increase of pressure on the time of flow is slightly less for blood at fever temperatures than for the same blood at lower temperatures.
7. The pressure influence is also greater for blood containing a large

number of corpuscles than for blood containing few (*cf.* curves in Curve-Table 9).

8. The addition of certain chemical reagents decreases the viscosity, whilst other substances may increase it.

An Indication of the Bearing of Viscosity upon the Circulation of the Blood.

In the next few pages we wish very briefly to indicate the import of such results as the foregoing in any consideration of the mechanism of the circulation of the blood. For this purpose we may with advantage regard the human circulatory system from a simple mechanical point of view, as a well-arranged though very complex net-like labyrinth of elastic pipes, namely, the arteries and veins, .connected with a central pumping-station, the heart, which maintains a constant circulation of a viscous fluid, having a peculiar and variable consistency—the blood; and to this simplified circulation endeavour to apply the above conclusions, when alterations occur affecting the whole or any one part of this system; for it is evident at once that any disturbance in the mechanism may arise from—

1. Conditions that interfere with the normal activity of the heart;
2. From alterations in the calibre of the blood vessels;
3. From changes in the amount or the composition of the blood with the consequent variations in viscosity.

Effect of Temperature.—For instance, it is well known that in ordinary healthy persons, as a result of vigorous exercise, the temperature may rise to 101° F., or even higher, and there is associated with this condition of hyperthermia an increased frequency of the heart beat, and at the same time dilatation of the peripheral blood vessels. The result of these changes is to increase the velocity of the blood flow: firstly, because the increased temperature will give rise in itself to a diminution in viscosity; and secondly, the increased calibre of the vessels reduces the peripheral resistance. As a nett result, there is a more rapid filling of the heart, but for each individual beat there is less resistance to be overcome, and consequently the heart is saved an appreciable strain; that is to say, we have a simple illustration of nature's way of mechanically adjusting the balance of the circulatory system so as to compensate for the extra work thrown upon the heart.

Passing from this normal physiological reaction we are led to consider the conditions met with in fever. In the early stages, with a raised temperature and a rapidly beating heart, there is an undoubted increase in the peripheral resistance (Maragliano)* and, moreover, the composition of the blood is altered—there being invariably a slight leucocytosis and an addition to the metabolic products contained in the circulating blood, which alterations in themselves tend to raise the viscosity.

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* Maragliano, 'Zeit. f. Kl. Med.,' vol. 14, p. 309, 1888.

Apart, therefore, from the raised temperature, these changes in the mechanism would tend to throw more work upon the heart, which, however, owing to its great reserve force, can respond for a time; fortunately in most cases, before it begins to flag, the second stage, or fastigium, is reached, in which the peripheral resistance is relaxed. This loss of vasomotor tone is always a noticeable feature at this period, and as a result the work of the heart is once more considerably lightened. Experimental evidence has given abundant proof of the marked increase in the rate of flow that follows upon a widening of the conducting channel; and further, our results have shown that with fine capillaries this influence is considerably greater than the fourth power of the radius, as is stated in Poiseuille's formula.

We would emphasise here again the marked influence of temperature on the viscosity of all fluids; for instance, the viscosity of water at 30° C. is one-half of what it is at 0° C., whilst with blood containing some $5 \cdot 10^6$ red corpuscles per cubic millimetre, our Tables, in some cases, show as large a change as 3 per cent. per 1° C.

Now the usual reasons given for the more rapid beating of the heart are: (1) the disturbance of the nerve centres; (2) the direct action of the patient's overheated blood upon the heart muscle. This last statement is founded upon the experimental ground that by perfusing an isolated mammalian heart with warm blood there results a more frequent contraction. Now we would add a third reason: *the more rapid filling of the heart* as a result of (1) the lessened resistance; (2) the diminished viscosity; therefore, as a consequence of the increased rate of flow, the diastolic period of the heart is lessened, and the ventricles refill much more quickly.

To quote Dr. Graham Brown, "a febrile temperature may be considered as a boon to the organism, in that it will allow the blood to circulate faster or it will save the work of the heart"; a deduction of the utmost importance, and one which, so far, has apparently received very little notice.

Effect of Change in Calibre of Vessels.

Again, alterations in the capacity and in the actual structure of the vessels are far from uncommon as the results of disease. The lesion which may follow an infection or intoxication is, unfortunately, not limited, but usually involves, step by step, the whole arterial tree. We may take as an instance of this condition the arterio-sclerosis which occurs in chronic nephritis; in these cases it is the smaller vessels that are attacked first and their lumen gradually encroached upon. We know that the rate of flow in capillaries is much slower than in the arteries and veins, for it is here that the greatest resistance is met with, and consequently most of the driving pressure is spent; if, therefore, the arteries leading to the capillary area are contracted in the slightest degree the sum total of the increased resistance in such an extensive change as occurs in Bright's disease must make a very marked difference in the work necessary to bring about a minimum circulation through the capillaries, that is to cause a blood velocity of the order of 0·5 mm. per second. We have already seen that the frictional resistance produced by alteration in the smaller capillary bores increases very rapidly with diminution in size, more especially with the finer periphery vessels. In order, therefore, that the mechanism be fully compensated for the narrowing of the smaller channels, there must be either: (1) an increase in the driving force, that is, a more powerful heart beat, or (2) a diminution in the viscosity of the blood itself. As is well known, the compensation in Bright's disease is usually brought about by an hypertrophy of the heart itself; and apart from any changes which may occur in the constitution of the blood (which indeed often shows an increased viscosity) we consider that the enormous increase in peripheral resistance occasioned by the general contraction of the smaller vessels affords an ample explanation of the vexed question of the cause of cardiac hypertrophy which is so truly necessary to bring about an adequate circulation. Furthermore, we do not see any reason to reinstate Bright's theory that there is some unknown substance circulating in the blood which acts directly on the heart muscle, as Hirsch and Beck have recently attempted.

Effect of Changes in the Composition of the Blood.—With regard to the changes in the composition and constitution of the blood, which will give rise to variations in the viscosity and, as a consequence, affect the circulatory mechanism, one can only remark here upon their infinite variety. Taking the plasma alone there may be (1) an increase or diminution in its volume; (2) proteid strength may be altered; (3) salts may be deficient or in excess; (4) metabolic products, *e.g.*, alloxuric bases and other nitrogenous derivatives abnormally abundant; (5) excessive saturation with gas, for instance CO_2; (6) presence of ferments, organised and unorganised. All these in themselves are capable of altering the viscosity values.

Or again, if we consider the corpuscles, viscosity changes can be produced by (1) variations in number of reds; (2) variations in number of whites; (3) variations in size.

We may here make mention of the following interesting experiments of Ferrai on the viscosity of the blood in partly asphyxiated animals. In his investigation with serum alone he found that the addition of CO_2, even to saturation, produced little or no change, whilst in the case of blood there was a noticeable increase in each addition of CO_2. This he attributed in part to the swelling of the corpuscles; his surmise was proved to be correct by measuring the volume of the corpuscles by means of the hæmatocrite (it was found to be increased).

Before we could make any investigation on the viscosity of the human blood it was necessary to devise an instrument capable of giving results with a small quantity of blood, since the instruments in use on the Continent required more blood than was obtainable or even permissible in this country from patients. Hirsch and Beck had been experimenting with 2 cm. of blood, whilst Dr. Parkes Weber's instrument requires even larger quantities of blood. Dr. Ewart had devised an instrument, but it was too complicated and required too many accessories for ward work.

Fig. 10 shows the form of instrument which we ultimately adopted and which is fully described in Appendix I.*

With this viscosimeter numerous observations have been made upon healthy individuals with fairly constant results, the average value found being roughly about five times that of water.

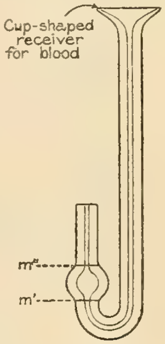

Fig. 10.—Clinical Viscosimeter for use with small quantities of Blood.

The following Table (VIII) contains viscosity values for the blood of five men in the best of health, with a record of their temperature and blood count.

Table VIII.—Viscosity Values in five Healthy People.

—	Temperature.	Blood count reds.	Time compared with water.
	°		
I	98·4	4·000·000	4·82
II	98·0	5·100·000	5·01
III	98·6	5·200·000	5·63
IV	98·4	5·500·000	5·21
V	98·4	5·700·000	5·42

Between the highest and lowest values in this table there is a difference of 0·81 and if we look at the blood count it is seen, with one single exception, that it rises with the number of the corpuscles present, the temperatures being almost identical. Hirsch and Beck, using much larger quantities of blood however, found with their viscosimeter that the time required by the blood of normal individuals to pass the tube varied to the extent of only 0·2 of seconds and concluded that the average viscosity might be put down as five times that of water.

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* We desire here to acknowledge our indebtedness to our colleague, Dr. Guy Barlow, for much kindly help, and for the suggestion that led to our making and using this type of instrument.

As indicating the influence of the number of corpuscles upon the viscosity values we give the subjoined tables; in the one (Table IX) is shown the effect of a diminution of the number of red cells and in the other (Table X) the effect of an increase.

Table IX.—Viscosity Values in Three Cases of Marked Chlorosis.

—	Temperature.	Pulse.	Respiration.	Blood count.	Viscosity values.
I	° 98·0	94	24	2×10^6	2·14
II	97·6	96	26	3×10^5	3·4
III	97·8	100	26	3×10^6	3·6

Table X.—Viscosity Values in Polycythæmia.

—	Blood count.	Viscosity values.	Observer.
I	$9·0 \times 10^6$	11·5	F. Parkes Weber*
II	$9·0 \times 10^6$	11·8	F. Parkes Weber
III	$6·0 \times 10^6$	6·5	F. Parkes Weber
IV	$8·3 \times 10^6$	9·4	J. H. Watson

With regard to the influence of an increase in the number of white cells we may quote the following observations made in the wards upon a patient suffering from advanced spleno-medullary leucocythæmia, having a blood count of 2·4 million red and 76,000 white corpuscles with a temperature of 98° F.; we obtained a viscosity value of 5·6 times that of water. Here we have an apparently normal viscosity value, but if we consider the decided falling off in the number of reds, and knowing that from that cause alone there ought to be a great lowering, we are justified in assuming that the maintenance of this high value is dependent in part at least upon the presence of the excessive number of the larger leucocytes.

The above instances have been quoted as simple illustrations of the influence that changes either in the blood, whether physical or chemical, or in the containing vessels may have upon the circulatory mechanism; numerous other examples might have been added; but for the present they will sufficiently serve to indicate how the work of the heart may be relieved or embarrassed by such variations. Moreover, we can thus see that the viscosity of the blood is not an independent but a dependent variable, which is essential for the maintenance of the series of adaptive changes that may occur in the circulatory system of the individual, be they physiological or pathological. And in concluding the communication we feel that the urgent need for further research in this field cannot be too strongly emphasised, in so far as even now we are still upon the borderland.

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* F. Parkes Weber, 'Brit. Med. Jour.,' January, 1906, p. 82.

Appendix I.

Description of Clinical Viscosimeter.

The viscosimeter which we have devised for clinical purposes consists simply of a curved piece of capillary tubing with two arms. The long arm, 6 cm. in length, has been blown out at its free end into a cup-shaped receiver with a thin edge. On the short arm, about 2 cm. in length, there is a small elliptical bulb and the point at which the capillary enters and leaves the bulb is etched on the glass (*vide* fig. 10, m' and m'').

To use the Instrument.—The most convenient place to take the blood for comparative estimations of the viscosity is, in our opinion, the lobe of the ear, for not only is it less sensitive than any other part, but it can be made to bleed readily without any manipulation, whilst the position of the hanging drop permits the viscosimeter to be placed vertically. The lobe of the ear is first well cleaned with ether and a special fine pointed knife is then inserted into the most dependent part of the lobe. The viscosimeter, which has previously been warmed to the temperature of the body, is now placed underneath the hanging drop of blood and the receptacle filled. The moving thread of blood is carefully watched through its course down the long limb and bend of the tube, a stop-watch is held in readiness during this time and the seconds finger started as soon as the column of blood reaches the point m', and stopped the moment the column gains m''. The time is now read off to a fraction of a second and the result compared with the reading for water. This time value is a reliable comparative indication of the viscosity of the blood under examination. For it follows from Poiseuille's law that if the length and diameter of the viscosimeter, the quantity of fluid, and the pressure height be kept constant, and the time be observed, then the viscosity of two liquids of densities s' and s'' with times of flow t' and t'' will be as

$$n' : n'' = s't' : s''t'',$$

and hence, if we neglect the differences of densities,

$$n'/n'' = t'/t'',$$

a result which should in general be correct to about 1 per cent.

In using these tubes the following points must be observed, otherwise serious discrepancies and much difficulty will be encountered.

1. The tubes ought to be scrupulously clean and after use are best cleaned as follows:—The blood is at once driven out by a small force pump in order that clotting may not occur in the tube. The viscosimeter is then filled with strong nitric acid and placed aside for a short time, the acid is next replaced in quick succession by distilled water, alcohol and ether, and the tube finally dried by blowing a current of air through it.

2. The viscosimeter should be previously brought to the temperature of the patient.

3. It is absolutely necessary to fill the receptacle with blood, for if the column of blood in the capillary breaks from the fact that there is an insufficiency, the experiment is utterly useless on account of the altered pressure height which the method presupposes constant.

4. It is a wise precaution to fix on to the short limb of the tube a small piece of rubber tubing, so that, if the blood should at first refuse to flow through the instrument, movement may be initiated by slight suction.

5. Determinations of the viscosity of the blood should be accompanied by a careful blood count and observation of the temperature.

6. In these estimations a good assistant will be invaluable, whose care it should be to time the rate of flow.

On the Myelins, Myelin Bodies, and Potential Fluid Crystals of the Organism.

By J. G. Adami, F.R.S., Montreal, and L. Aschoff, Marburg.

(Received June 6,—Read June 7, 1906.)

(From the Pathological Institute of the University of Marburg.)

When, in 1854, Virchow recognised myelin as, if not a single substance identical with the essential constituent of the medulla of nerves, at least as a class of substances which chemically were closely allied thereto, he described it as possessing the following characteristics :—

(i) When brought into contact with water it swells up, exhibiting a characteristic morphology, and developing processes of irregular and bizarre form having a double contour.

(ii) Easily soluble in hot alcohol, becoming in part precipitated on cooling.

(iii) Rapidly dissolved in ether, chloroform, and turpentine.

(iv) Acted upon but slowly and to a slight extent by weak acids and alkalies.

(v) Undergoing shrinkage under the action of strong alkalies, with loss of characteristic properties.

(vi) Swelling greatly under the action of concentrated sulphuric and other strong acids, with eventual dissolution.

Substances possessing these properties to a greater or less extent, and especially the property of swelling in water and exhibiting "myelin forms," have been recorded during the last 50 years as occurring in very many organs, and these either under physiological or under pathological conditions. How widespread they are is indicated by the following table, which we give because we have not met with any attempt at a full classification in the recent literature of the subject :—

I. *Intracellular Myelin Bodies.*—These are small, highly refractile bodies situated within the cytoplasm, which, under the action of water or physiological salt solution, swell up and assume irregular forms. According to the conditions under which they are found they may be redivided into—

A. *Physiological,* or associated with normal regressive processes, in—

The suprarenal cortex of man and of several vertebrates (Kaiserling and Orgler).

The granular cells of the corpora lutea (Do.).

The thymus gland (Do.).

B. *Pathological.*—

Fatty patches of the aortic endothelium (Do.).

Atheromatous areas in the arterial walls (Mettenheimer, Torhorst).

Lungs: Alveolar epithelium of the new born (Hochheim), and of adults (Wagner), lung tissue in general in various diseased states (Virchow, Mettenheimer, etc.), bronchial epithelium (Schmidt, Kaiserling, and Orgler).

Kidney: Fatty degeneration (Albrecht, Löhlein and, in certain cases only, Kaiserling and Orgler), cells of renal tubules after arterial ligature (Albrecht).

Crystalline lens in both soft and hard cataract (Mettenheimer).

Tumours; cells of many tumours, cancerous and sarcomatous (Kaiserling and Orgler).

C. *Autolytic*, developing *post-mortem* in the aseptically preserved organ.

Lung, alveolar epithelium (Albrecht, Hochheim).

Kidney and liver cells (Albrecht, Waldvogel, Dietrich and Hegel, etc.).

Skeletal and heart muscle (Dietrich and Hegel).

Here may be included possibly the myelin bodies seen often in morning sputum (Müller and Schmidt), developed in dissociated cells. The cases of appearance of myelin bodies in the cells of necrosed areas would appear to form a connecting class between B and C.

II. *Diffused Myelin.*—Myelin not recognisable in the tissues in the form of discrete bodies, but gained from them by digestion with alcohol.

Here are included myelin substances from the brain and nerve tissue (Berzelius, Drummond, Virchow, Gobley, Liebreich, etc.), egg yolk (Gobley, Virchow), spleen, suprarenal medulla and colloid thyroid (Virchow), blood (Gobley), mesenteric chyle (Beneke), red corpuscles (Albrecht), pus (Virchow). Indeed, most organs if digested with absolute alcohol at 40° to 45° C. will yield lecithin-like substances which become precipitated in part upon cooling to 0° C. and in various fluids afford well-marked myelin forms. And, as pointed out 44 years ago by Beneke, this form of myelin is widely distributed throughout the animal and vegetable kingdoms.

III. *Myelin-like Substances in Secretions.*—Bile of gall bladder (Virchow). Contents of small intestine after fatty meal (Beneke).

Leaving aside the second class, in which it would seem that we have to deal with impure lecithin (for lecithin purified from cholesterin does not, we find, exhibit marked myelin formation), it is obvious from the descriptions given of the microchemical characters, either that there are several bodies possessing myelin properties, or, if only one body, that then it exists in very varying degrees of purity, for the reaction with osmic acid varies, as does that with dyes such as Sudan III, neutral red, saffranin and methylene blue. In one and the same specimen these reactions may vary.

A very characteristic property of a large number of myelin bodies (shown, we find, very imperfectly by the autolytic group, at least in the early stages of autolysis) is that of *double refraction.* If a section or some of the expressed juice of the suprarenal be taken, what under the ordinary microscope appear to be minute fatty globules are seen in part to exhibit double refraction under polarised light. Between the crossed Nicol's prisms each little globule exhibits a black cross with four highly illuminated, bright and colourless intervening sectors. This property was first observed by Mettenheimer in 1858, rediscovered by Apathy for nerve myelin in 1890, by Müller and Schmidt in 1898 for sputum myelin and again, independently, by Kaiserling and Orgler for suprarenal myelin in 1902. The question whether the presence of doubly refracting myelin globules in atheromatous vessels as noted by Torhorst in the laboratory of Aschoff could be reconciled with Klotz's detection in Adami's laboratory of compound soaps in the same areas led to the present investigation.

The following bodies gave negative results: pure fatty acids, the glycerides of fatty acids (neutral fats), pure lecithin,* pure protagon, cholesterin, sodium, potassium and calcium stearates and palmitates, spermin plus oleic acid. Under no conditions of heating or employing various menstrua could we with these obtain doubly refracting globules.

The following bodies gave positive results: the oleates of sodium potassium, ammonium and calcium; the stearate and palmitate of ammonium; cholesteryl oleate, palmitate, stearate, and butyrate (obtained by heating together cholesterin and one or other fatty acid), lecithin plus cholesterin (? cholesteryl lecithate); cholin oleate; lecithin acted upon by ammonia, caustic potash, and caustic soda. With all these substances by one or other means it was possible to gain perfect small spherules, highly refractive, affording the characteristic cross when examined between Nicol's prisms.

It was clear, therefore, that, *inter alia, certain bodies of the nature of soaps afford this particular reaction.* But at the same time, with not a few of these bodies, notably the simple soaps and the lecithin compounds with which the investigation was begun, we could not determine with accuracy what were the factors leading to the successful production of the globules. Of two preparations made apparently alike and subjected to like treatment, one might present them, the other not. Often, notably in the case of the stearic and palmitic acid compounds, the phenomenon was transient, not to say momentary. After heating the two components together to form the salt (or soap) and this in the presence of water or weak alcohol, upon examining the slide as it cooled, the globules might flash out, sometimes in a perfect rain over the field, and rapidly give place to a general crystallisation. Globules which cannot persist at the room temperature cannot be taken into consideration as being possibly associated with the doubly refractive myelin bodies of the human organism.

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* The form employed was Riedel's lecithol: the so-called pure lecithin from egg yolk contains abundant cholesterin.

The stearic and palmitic compounds are thus seen to be placed aside. But even with the simple oleates, potassium oleate, for example, while globules might be present even after several days, in other preparations they might be wholly wanting, being replaced by crystals. This with the simple soaps was the rule and not the exception.

These uncertainties, coupled with the evident relationship of the globules to the process of crystallisation, and the observation that the condition in the atheromatous aorta disappeared upon heating, to reappear upon cooling, led one of us to consult his colleague, Professor R. Schenck, regarding the nature of these globules, and, as it happened, he consulted one of the authorities upon the subject. As the work of Lehmann and Schenck is evidently as little known by other pathologists and biologists as it was to us, a short notice of their observations is imperative. Briefly, these doubly refractive globules are "fluid spherocrystals." The present status of knowledge and opinion regarding these is given clearly and succinctly in Schenck's 'Flüssige Kristalle' (Leipzig, Engelmann, 1905). We would here thank Professor Schenck heartily for his aid in this and other matters.

The impression that a crystal is a solid unyielding body cannot be maintained. It has been known for long that metals like lead and gold can, under pressure, be forced through apertures, as also with ease can solid sodium. The earlier view was that we had to deal with the gliding of one solid crystal upon the other. Lehmann's observations, extending over more than fifteen years, have shown that there exist substances which, when heated, pass into an intermediate state. From being solid they become fluid, but still, under polarised light, they are seen to maintain the characteristic crystalline feature of being doubly refractive or at least of containing doubly refractive bodies. Some of these fluids are thick like olive oil, some, as for instance *para*-azoxyphenetol, are more fluid than water. Heat to a further degree, and the fluid becomes perfectly isotropic without a sign of double refraction.

In this intermediate phase we deal with crystalline fluids, and the individual crystals are fluid crystals, capable of being distorted by slight pressure. We will not enter into the distinction between flowing, or ductile, and fluid crystals, for the difference is purely one of degree. Nor is it necessary here to discuss the opposing theories of Tammann and Quincke, that the phenomenon is essentially due to the presence of two substances, the truly fluid matrix and the suspended crystalline body. Schenck has demonstrated by exact physical methods that it is capable of occurring with absolutely pure bodies of this order. When the substances of this nature are suspended in an inert matrix, upon passing from the solid to the intermediate state or on being cooled from the isotropous to the intermediate state the separate globules assume the form and characters of anisotropic fluid spherocrystals.

Towards the end of 1905 some 20 substances of this nature had been described. The majority of these are para-derivatives of anisol and phenetol, with melting points considerably above 100° C. and "clearing points" (when they become isotropic) from 20° to 300° C. higher (this last in the case of silver iodide). Of more immediate interest is a series of *acid esters of cholesterin*—cholesteryl oleate, cholesteryl benzoate, cholesteryl propionate—and, for our purposes, of yet greater interest is the *oleic acid group.* The cholesteryl soap we have already mentioned; Schenck reports also the Na, K, and NH_3 oleates, dimethyl and trimethyl NH_3 oleates. These have not been so fully studied; the very weakness of olein as an acid renders it difficult to obtain the substances in a state of absolute purity, free from dissociation products. Hence their "melting" and "clearing" points have not been determined.

Altogether, towards the end of 1905, some 20 of these crystalline fluids (or potentially crystalline fluids) had been described. Their number is being rapidly increased. Lehmann, in one of his test-papers, mentions one observer as contributing a series of 10 new bodies. It will be seen that, employing the formation of anisotropous spherocrystals as an index, we add a considerable number—ammonium stearate and palmitate, cholesteryl stearate, palmitate and butyrate, calcium oleate (?),* cholin oleate and possibly the lecithates of K, Na, NH_3 and cholesterin. We mention these last tentatively. Very possibly these bases dissociate the fatty acids from the lecithin, and the spherocrystals seen by us are merely dissociation products combined with the introduced base.

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* We have here some doubt, but believe that the crystals here pass through a fluid, perfectly globular state, before passing on to the solid spherolith state, which they are apt to assume.

One striking feature is possessed by all these crystalline fluids, that, namely, of dissolving other substances and still undergoing crystallisation. Ordinary crystalline substances can only dissolve substances that are strictly isomorphous; these can dissolve other fluid-crystalline substances in all proportions and have a generous capacity for dissolving substances of other orders also. This fact bears intimately upon the varying microchemical reactions exhibited by myelin bodies even in the same specimen. This dissolving power alone is of high significance if, as indicated, diffused myelin, or myelins of lecithin-like nature, are essential constituents of the cells of most tissues. The lecithin, present in abundance in the red corpuscles, well fits them to be the common carriers of the organism.

Associated with this is the fact that admixture lowers the melting and the clearing points; so that of two globules of myelin, one containing admixed more fatty acid or neutral fat than the other, the one may present itself as a strictly fluid globule—isotropous—the other as a crystalline fluid globule—anisotropous. Similarly, the surrounding menstruum has a profound effect. We found with cholesteryl oleate that, using water as a menstruum, we could not by any means gain spherocrystals; at Professor Schenck's advice we employed, with success, a minimal amount of dilute alcohol; using a larger quantity, the result was that the compound remained for days completely isotropous, only as the alcohol slowly dissolved out of the oily mixture did spherocrystals appear at the periphery. It may here be noted that pure cholesteryl oleate is in the intermediate state at room temperature (15° C.), presenting somewhat elongated prism-like crystals which, upon pressure, alter their form, regaining it when the pressure is removed. The same is true of cholin oleate.

These properties explain the difficulties encountered in developing spherocrystals in our earlier experiments; a slight excess of one or other constituent of the soap or change in the relative amount of the menstruum materially influences the development and appearance of the spherocrystals. We are still uncertain whether pure lecithin forms spherocrystals; it has all the characters of a crystalline fluid; but for now many months attempting a great number of expedients, we have uniformly failed to develop the globular anisotropous state.*

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* Here it may be laid down that the formation of myelin processes and of spherocrystals, which, it is true, often exhibited by the same substance, are not necessarily correlated. A substance may show myelin forms and not be able to exhibit spherocrystals and *vice versâ*.

What, then, is myelin? We are still unable to state definitely, but believe that the considerations and observations here brought forward lead one step nearer to the solution of the problem. Various conclusions have been reached, from Beneke (who regarded it as a glyceride of cholesterin, and laid down that "ohne Cholestearin keine Myelinformen"), through Liebreich (who held that while protagon itself might not exhibit myelin forms it would probably be found that where myelin forms developed, there protagon was constantly present), to modern workers upon autolysis (who ascribe the phenomena to lecithin), and lastly, to those who, with Neubauer and Quincke, regard myelin formation as the expression of a physical state common to a very large number of bodies.

There is one significant fact in this connection, namely, that, *so far, the only crystalline fluids known which are in the intermediate state at the room temperature are certain of the oleic acid compounds;* in fact, it may be laid down from our observations that almost all the simpler oleates and some like lecithin, of the more complex are in the fluid-crystalline state at 15° C Lecithin has definitely been determined to be the main constituent of the diffused myelin of normal tissues, and it is interesting to note that all the bodies, with the exception of cholesterin, which have been adduced as the essential constituent of myelin—protagon, lecithin, soaps—are oleic acid compounds, and, as regards cholesterin, Beneke's experiments indicate that he dealt with cholesteryl oleate. It may be that it is wrong to include lecithin in this group. There exist in the organism di-stearo- and stearo-palmito-lecithins, and these are more abundant than is di-oleo-lecithin. While many considerations indicate that oleic acid compounds in particular possess properties most allied to those of the myelins of the organism, we cannot thus absolutely exclude the other fatty acids. We may, however, it would seem, confidently conclude that *fatty acid is an essential constituent of "myelin,"* and that of the fatty acids oleic acid *plays the most important part.*

Is it possible to proceed any further? The evidence at our disposal appears to point to at least two different varieties of myelin, one in which cholesteryl oleate is the essential constituent, another in which we have essentially to deal with cholin oleate.

Examining the aorta of a middle-aged man, we found in the thoracic portion a single atheromatous area, affecting the middle as well as the inner coat. This afforded a small amount of dry atheroma material. Upon making a smear of this, while it showed abundant and large plates of cholesterin, and abundant fatty globules, there was not a single doubly refracting globule present. Upon warming the slide gently over the flame and examining when cool, there were now abundant anisotropous globules, with typical black crosses and alternating bright quarters. These occurred more particularly in the neighbourhood of and lying over the cholesterin plates, which showed some signs of erosion. The indications pointed to the conclusion that here cholesterin had been taken up by the fatty globules which then became anisotropic.* We have repeatedly noted that after warming specimens—smears—from the atheromatous aorta, the spherocrystals which had been colourless became prismatic, and this prismatic colouring is characteristic of cholesterin compounds. Virchow's observation, confirmed by Beneke, regarding the existence of "myelin" in the gall bladder is significant, and when to this we add Hürthle's demonstration that cholesteryl oleate is a constant constituent of the blood, and that of Aschoff that fat is absorbed actively by the epithelium of the gall bladder, thus affording the most satisfactory explanation yet given of the development of cholesterin calculi—cholesteryl oleate being excreted and becoming dissociated—we gain strong ground for believing that cholesteryl oleate is a more important factor in myelin formation than has hitherto been held. If we cannot accept in its entirety Beneke's dictum "ohne Cholestearin keine Myelinformen," we recognise that his view, so long discredited, gains a measure of support, at least for some instances of myelin formation.

The case in favour of cholin oleate is more novel. So far as we are aware, this substance has not hitherto been isolated. Liebreich, in 1865, came close to making the discovery, but was misled by his prepossession in favour of protagon, which he was the first to isolate. He then showed that (1) protagon alone does not produce myelin forms, (2) protagon with oleic acid (with which it readily mixes) also gives negative results, (3) a trace of neurin (cholin) added to the above mixture results in the production of exquisite myelin figures. And he concluded that bodies, like protagon, which have the power of swelling in water and of being dissolved in fatty acids, develop myelin forms when dissolved in the respective soaps of these acids. The neurin, he pointed out, must here form a soap with the excess of fatty acid, just as did Na, K, or NH_3, which gave the same myelin formation. The fourth experiment, to complete the series, he never attempted. Had he done so he would have found that the presence of protagon is not essential, that cholin combines with oleic acid in the cold, forming immediately a thick crystalline fluid, a drop of which placed in water forms most extraordinary myelin figures, more extraordinary even than those of ammonium oleate, long processes being rapidly thrown out, which coil themselves into most complicated knots. These processes are doubly refracting. By slightly altering the conditions of the experiment abundant spherocrystals are formed.

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* As confirming Klotz's observations upon the differential staining of neutral fats and soaps with Sudan III, it may be noted that in another atheromatous aorta we found that the globules which were doubly refractive assumed a paler stain with this dye, the fatty isotropous globules a deeper red.

Here, then, we have a nitrogenous base, a product of proteid dissociation, uniting with oleic acid to form a soap. We reserve a fuller description of the properties of this body for another occasion. We would only point out that cholin is a constituent of both protagon and lecithin: that neither of these, it would seem, afford myelin formation in the pure state: both are unstable and that largely because of the chemically weak fatty acids which enter into their composition. When, therefore, these bodies are found associated with myelin formation it would seem most probable that the dissociation of the combined cholin permits it to act upon the oleic acid also present and that this is the essential cause of the myelin formation in this group of cases.

There is still, we believe, some debate as to whether the neurin of Liebreich is identical with cholin or no. Employing a solution of "neurin" (Merck), it may be noted that we gained like results.

Lastly it has to be noted that there exist solid, doubly refractive crystalline bodies (or spheroliths) as well as these fluid spherocrystals. They are of two forms, that of solid spheres and that of spherical masses of closely packed, radiating, fine acicular needles. The appearance under the ordinary microscope serves to distinguish these from the fluid crystals; they lack the oily appearance of the latter, and even the most spherical of the first class are apt to be nodose on the surface, or to occur in pairs, as with some of the calcium salts, or to be distinctly irregular in shape. Both forms may occur in the organism and both between the Nicol's prisms give double refraction. With the radiate form there is little chance of confusion; of the more globular form the most frequent example is encountered in the uratic deposits in the renal collecting tubules forming the uratic "infarcts" of the new born. In our own observations we have obtained exquisite and abundant radiate spheroliths by the action of weak formaline upon cholin oleate (after several hours): of the small globular forms calcium oleate affords excellent examples.

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Note during Correction.—While this paper has been passing through the press, we have come across an article by O. Stoerk ("Ueber 'Protagon' und über die grosse weisse Niere," 'Sitz.-Ber. d. Kais. Akad. d. Wiss. in Wien,' Math.-Naturwiss. Kl., vol. 115, Abth. 3, February, 1906, 1), in which, from a consideration of certain analyses by Panzer, the author concludes that the myelin of the chronically inflamed kidney is most probably of the nature of a compound of fatty acid and a cholesterin ester. Other myelins (although he does not employ the term) he regards as possibly of the nature of protagon.

The Alcoholic Ferment of Yeast-juice. Part II.—*The Coferment of Yeast-juice.*

By Arthur Harden, D.Sc., Ph.D., and William John Young, M.Sc.

(Communicated by Dr. C. J. Martin, F.R.S. Received June 14,—Read June 28, 1906.)

(From the Chemical Laboratory, Lister Institute.)

In a previous communication[*] it was shown that the fermentation of glucose by yeast-juice is dependent upon the presence of a dialysable substance which is not destroyed by heat. This substance is contained in the active yeast-juice prepared by disintegrating living yeast, and, therefore, most probably exists in the yeast cell side by side with the zymase.

The occurrence of an analogous activating substance has been described by Magnus[†] in the case of the lipase of the liver. He observed that the active juice of this organ became inactive when dialysed into water, but regained its activity when the dialysate or boiled liver juice was added. The term *coferment* was suggested by Bertrand[‡] to denote substances of this kind, and he applied it in two instances—to the calcium salt, which he considered was necessary for the action of pectase on pecten substances, and to the manganese of laccase, which he supposed to be essential for the activity of this enzyme. Although not entirely satisfactory, this term may be provisionally applied to activating substances such as those present in liver lipase and yeast-juice, until further knowledge of their nature and function permits of a more rational terminology.

(1) *Preparation of the Inactive Residue from Yeast-juice in the Dry State.*

In the previous communication[§] it was shown that when yeast-juice is filtered through a Martin gelatin filter, both the residue and the filtrate are incapable of fermenting sugar, whereas a mixture of the two produces a vigorous fermentation.

In carrying out the experiments which established this result, the residue left on the filter was always dissolved in water as soon as the filtration and washing were completed, and the activity of the solution was examined without delay. It has since been found possible to obtain the inactive residue in the solid form, in which condition it retains its properties for a considerable time.

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[*] 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 410.

[†] 'Zeit. Physiol. Chem.,' 1904, vol. 42, p. 149.

[‡] 'Compt. Rend.,' 1897, vol. 124, p. 1032.

[§] *Loc. cit.*

This is accomplished by spreading the sticky mass left on the filter over a large watch-glass, and exposing it over sulphuric acid in a vacuum. The residue dries up in a few hours to a brittle mass, which is converted by grinding into a light yellow powder.

Complete removal of the coferment is generally not effected by a single filtration, and the powder prepared as above is usually found to be slightly active. A completely inactive residue may, however, be obtained by redissolving in water and repeating the filtration and desiccation. The powder prepared in this way and dried over sulphuric acid in a vacuum for 15 hours only loses its potential activity slowly. The following examples show the original inactivity of the powder, its fermentative power in presence of boiled yeast-juice, and the loss of this power with lapse of time:—

I. 200 c.c. of yeast-juice were filtered in quantities of 50 c.c. through Martin filters and yielded 17·6 grammes of dry solid. This was dissolved in 50 c.c. of water and again filtered, yielding 9·2 grammes of dry solid residue.

The following experiments were then made at 26° in presence of toluene:—

1. March 8, 1906. One gramme of dry residue was dissolved in 15 c.c. of water and 1·5 grammes glucose added. No evolution of carbon dioxide was observed; 12 c.c. of boiled yeast-juice containing 1·2 grammes of glucose were then added. Fermentation at once commenced, and 108 c.c. of carbon dioxide were evolved in 20 hours, when the experiment was discontinued.

2. Two months later (May 18, 1906) a similar experiment was carried out, a sample of the same boiled yeast-juice being employed. As before, 1 gramme of the residue dissolved in sugar solution was quite inactive. On the addition of the boiled juice fermentation at once commenced; the rate was, however, only about one-third of that attained in the previous experiment, and fermentation ceased when only 42 c.c. of carbon dioxide had been evolved.

Although the fermentation in the first experiment was not continued to the end, the comparison shows clearly that the potential activity of the dry residue had greatly diminished.

II. In the case of another sample of dry residue, with which two similar experiments were made with the interval of a week, it was found that in the first experiment 364 c.c. of carbon dioxide were produced, and in the second 344 c.c., a difference of only about 5·5 per cent.

(2) *Disappearance of the Coferment from Yeast-juice during Fermentation and Autolysis.*

When a small amount of boiled yeast-juice is added to a solution of the inactive residue in 10-per-cent. glucose solution, fermentation commences and continues for a period which varies with the amount added. The cessation of fermentation appears in such a case to be due to some change in the coferment, since the addition of a further quantity of boiled yeast-juice again sets up fermentation, and if the quantity of boiled juice added on each occasion be small enough, this process can be repeated a third time.

The following experiments, made at 26° in presence of toluene, illustrate this point:—

1. One gramme of dry inactive residue was dissolved in 15 c.c. of 10-per-cent. glucose solution, and three quantities of 3 c.c. of boiled juice were then successively added, the fermentation being allowed to come to an end before each addition.

	Carbon dioxide evolved.
1st addition …………	8·2 c.c.
2nd „ …………	6 „
3rd „ …………	6 „

2. Two grammes of dry inactive residue were dissolved in 15 c.c. of 10-per-cent. glucose solution, and two quantities of 15 c.c. of a diluted boiled juice containing 10 per cent. of glucose were successively added.

	Carbon dioxide evolved.
1st addition……………	54 c.c.
2nd „ ……………	41·2 „

This phenomenon has been frequently observed in the course of experiments on the filtration of the juice through the Martin filter. The first residue obtained is generally slightly active, and produces a small amount of fermentation with glucose solution. The evolution of carbon dioxide, however, soon stops, but is again renewed when boiled yeast-juice is added. One instance of this was quoted in our previous communication,* and many more have been observed, some of which are tabulated below.

Carbon dioxide evolved from residue + glucose.	Boiled juice added.	Subsequent evolution of carbon dioxide.
c.c.	c.c.	c.c.
8·6	20	72
13·2	16	364·3
16·4	20	368·8

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* *Loc. cit.*, p. 410.

The cessation of fermentation in any particular mixture of inactive residue and coferment may, therefore, be due to the disappearance either of ferment or coferment from the liquid. If the amount of coferment present be relatively small, it is the first to disappear, and fermentation can then only be renewed by the addition of a further quantity, whilst the addition of more ferment produces no effect. If, on the other hand, the amount of coferment be relatively large, the inverse is true; the ferment is the first to disappear, and fermentation can only be renewed by the addition of more ferment, a further quantity of coferment producing no effect. This is illustrated by the following experiment made at 26°:—

Two solutions were made up, each containing 0·5 gramme of dry inactive residue +10 c.c. of 10-per-cent. glucose solution and toluene, and to each of these 2 c.c. of boiled juice containing 10 per cent. glucose were added. As soon as fermentation had ceased, 0·5 gramme of dry residue in 10 c.c. of glucose solution was added to one solution (*a*), and 2 c.c. of boiled juice diluted to 10 c.c. with glucose solution to the other (*b*). Two other solutions were also prepared, each containing 0·4 gramme of the same dry inactive residue +10 c.c. of 10-per-cent. glucose solution + toluene, and 25 c.c. of the same boiled juice containing glucose were added to each.

As soon as fermentation had ceased, 0·4 gramme of residue dissolved in 10 c.c. of glucose solution was added to one (*c*), and 10 c.c. of boiled juice containing glucose to the other (*d*). The following were the results:—

	Boiled juice added.	Fermentation produced.	Subsequent addition.	Additional fermentation.
	c.c.	c.c.		c.c.
(*a*)	2	9·4	0·5 gr. residue	0
(*b*)	2	7·6	2 c.c. boiled juice	8·9
(*c*)	25	56·8	0·4 gr. residue	36·8
(*d*)	25	50	10 c.c. boiled juice	0

(3) *Rate of Disappearance of the Coferment from Yeast-juice.*

The following experiments were made to ascertain approximately the rate at which the coferment disappears from yeast-juice both in the absence and presence of added glucose. For this purpose a quantity of yeast-juice was preserved at 25° in presence of toluene, and samples were removed, boiled and filtered, at the commencement of the incubation and after various intervals. In one case a parallel experiment was made with yeast-juice to which 10 grammes of glucose per 100 c.c. had been added. The filtrates were then all rendered neutral to litmus and tested with equal quantities of an inactive residue and glucose, care being taken to keep the concentration of glucose the same throughout.

Material digested.	Time of digestion.	Volume of filtrate taken.	Fermentation produced.
	days.	c.c.	c.c.
1. *a.* Yeast-juice alone	0	20	168·5
b. „	9	20	0
2. *a.* „	0	16	364·3
b. „	2	16	2·6
c. „	4	16	0
3. *a.* „	0	15	62·9
b. „	1	15	2·1
c. „	2	15	0
d. „	4	15	0
e. Yeast-juice + glucose	0	15	62·9
f. „	1	15	15·6
g. „	2	15	4·5
h. „	4	15	8·3

In two cases in which a negative result was obtained (1*b* and 2*c*) an equal volume of the same fresh boiled juice was added and in both cases a vigorous fermentation was produced, thus proving that the autolysed juice had not exerted any specific detrimental effect on the ferment:—

	Fresh boiled juice added.	Carbon dioxide evolved.
1. *b*.	20 c.c.	112 c.c.
2. *c*.	16 „	297 „

It appears from this that the coferment disappears from the juice less rapidly in the presence of glucose than in its absence. In yeast-juice to which no addition of glucose has been made the coferment usually disappears at 26° in about 48 hours; in the presence of 10 per cent. of glucose coferment was still present at the end of four days, although only in a small amount. (The observation that the fermentation after four days' incubation is somewhat greater than that given after two days is probably to be explained by the presence of a greater amount of phosphate in the juice which had been digested for the longer period.)

4. *Soluble Phosphates do not Render the Inactive Residue Capable of Fermenting Glucose.*

In view of the fact that soluble phosphates, as described in the previous communication, exert a remarkable effect on the fermentation of glucose by yeast-juice, experiments were made to ascertain whether the addition of a soluble phosphate to a solution of the inactive residue in glucose is sufficient to set up fermentation. All the attempts hitherto made to effect this have yielded entirely negative results, although both the kind of phosphate and the amount added have been varied.

Dipotassium hydrogen phosphate, a mixture of this with the dihydrogen phosphate, disodium hydrogen phosphate, diammonium hydrogen phosphate, microsmic salt, and a mixed phosphate of potassium and magnesium obtained by boiling a solution of potassium dihydrogen phosphate with magnesium carbonate, were employed, all of which are capable of producing the characteristic effect of phosphates on the fermentation of glucose by yeast-juice.

Although these substances did not set up fermentation when added to an inactive filtered residue and glucose, they did not affect the potential activity of the residue. This is evident from the fact that the subsequent addition of boiled juice produced an immediate fermentation.

In every case the solution of the phosphate was saturated with carbon dioxide at 26° and added to the solution of the inactive residue in glucose solution also saturated with carbon dioxide at 26°, and in no case was any evolution of gas observed after the cessation of the slight disturbance which inevitably occurs when the solutions are mixed. The phosphate solutions were all of 3/10 molar strength, with the exception of the mixed phosphate of magnesium and potassium, 5 c.c. of which yielded 0·1594 gramme $Mg_2P_2O_7$.

In those cases in which the residue employed was slightly active, incubation was continued until all fermentation had ceased before the phosphate solution was added, and the amount of carbon dioxide evolved in this preliminary period is given in the table. The amount of glucose, both in the solution of the residue and in that of the phosphate, was throughout 10 grammes to 100.

	Amount of residue.	Preliminary fermentation.	Phosphate added.	Carbon dioxide produced.	Subsequent evolution of carbon dioxide after the addition of boiled juice.
1	25 c.c.	41·2	2·5 c.c. KMg phosphate ...	0	18·4
2	25 „	47·7	5 „ „ ...	0	—
3	25 „	0·6	3 „ K_2HPO_4	0	268·8
4	1·6 gr.	21·5	3 „ KMg phosphate ...	0	159·2
5	1·2 „	10·7	5 „ „ ...	0	—
6	0·5 „	0	2 „ mixture of K_2HPO_4 and KH_2PO_4	0	3 c.c. per hour
7	0·5 „	0	2 „ $(NH_4)_2HPO_4$	0	4·2 „ „
8	0·5 „	0	2 „ $(NH_4)NaHPO_4$ ...	0	3·8 „ „
9	0·5 „	0	2 „ Na_2HPO_4	0	3·6 „ „

The foregoing conclusion is confirmed by the observation, made in the experiments on the disappearance of the coferment from yeast-juice, that boiled autolysed juice does not set up fermentation in a mixture of the inactive residue with glucose, although it itself contains a large amount of phosphate precipitable by magnesia mixture. The following numbers were obtained by the analysis of three specimens of boiled autolysed juice employed in those experiments, two of which were quite inactive, whilst the other only produced a fermentation of 2·6 c.c.

No. of experiment.	Volume of juice.	Carbon dioxide evolved.	Phosphate present in grammes of $Mg_2P_2O_7$.
	c.c.	c.c.	
1. *b*	20	0	0·3400
2. *b*	16	2·6	0·3011
3. *c*	15	0	0·1893

These experiments throw no light on the actual chemical nature of the coferment, but show that most probably it does not consist of a phosphate precipitable by yeast-juice. They also indicate that substances, which, like phosphates, increase the total fermentation produced by yeast-juice, are not necessarily capable of setting up fermentation when added to a mixture of inactive residue and glucose.

Further experiments are in progress with the hope of obtaining more information on these points.

Studies on Enzyme Action.—Lipase : II.

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Author's title slip :—D. Q.
Subject slips :—
D 7090 Q 1230 Hydrolysis by Lipase.
D 8014 Q 1240 Ricinus and Liver Lipase.]

The following account has reference to the pursuance of the inquiry into the nature of the process whereby the fats are hydrolysed under the influence of lipase*; the experiments have been made in the hope of discovering an explanation of the selective power which the enzyme undoubtedly displays, as it promotes by preference the hydrolysis of ethereal salts of the higher fatty acids such as are present in the natural fats.

The active material used, as a rule, has been the finely ground air-dried residue which is left on depriving crushed castor oil seed of oil by means of ether; although but slightly active towards ethereal salts other than fats, it is easily prepared of uniform quality and does not soon deteriorate when kept. In all cases, the effect produced in blank control experiments is allowed for. As a rule hydrolysis took place at the temperature of the laboratory.

Operating with what appear to have been highly active materials, Kastle and Loevenhart† were able by means of hepatic and pancreatic lipase to effect the hydrolysis of several ethereal salts derived from the lowest terms of the acetic series of acids. The results they obtained in two series of experiments are as follows, the figures representing the percentage amount of salt hydrolysed after 15 minutes at 40° :—

Ethylic formate	1·60	1·75
" acetate	0·93	1·75
" propionate	1·05	2·87
" butyrate.........	3·13	4·37

As the formate is readily attacked by water alone, they regarded the values for this salt as high; unfortunately they made no control experiments with any of the salts. They came to the conclusion that the higher the molecular weight of the acid, the more readily is its ethylic salt hydrolysed by lipase.

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* Part I, see 'Roy. Soc. Proc.,' B, vol. 76, p. 606.

† *Loc. cit.* We have not yet had the opportunity of consulting Kastle's recent publication.

Ricinus lipase, at least in the form in which we have used it, has a very slight action on ethylic acetate, but gradually hydrolyses the butyrate. Alone it produces little or no effect, mixtures of 2 grammes of seed residue, 3 c.c. ethylic butyrate and 200 c.c. water requiring for neutralisation at the end of 21 hours only 0·7 c.c., after 45 hours 1 c.c. and after 117 hours 1·75 c.c. of normal alkali.

In presence of dilute N/5 acetic acid, action takes place slowly, as shown in the following table—in which the values represent the number of cubic centimetres of normal alkali neutralised:—

	A. 2 grs. seed residue. 20 c.c. acetic acid. 200 c.c. water.	B. 3 c.c. ethylic butyrate. 20 c.c. acetic acid. 200 c.c. water.	C. 2 grs. seed residue. 20 c.c. acetic acid. 200 c.c. water. 3 c.c. ethylic butyrate.
21 hours ...	0·18	0·2	3·2
45 „ ...	0·20	0·2	7·02
69 „ ...	0·20	0·2	10·10
112 „ ...	0·20	0·25	15·3

The extent to which hydrolysis is effected depends both on the amount of enzyme and, up to a certain point, on the proportion of acid present; thus the following results were obtained, using *x* grammes of seed residue, 0·4 c.c. toluene, 1·5 c.c. ethylic butyrate, *y* c.c. N/5 acetic acid, water to 100 c.c.

Seed residue.	Cubic centimetres of acetic acid.	Cubic centimetres of alkali required by acid liberated after—		
		19 hours.	43 hours.	67 hours.
gramme. 1	2	3·5	8·5	13·0
1	5	6·5	11·5	16·0
1	10	7·0	13·0	16·5
1	20	9·6	19·5	
0·5	3	3·0	5·0	7·5

The extent to which the activity of the enzyme is influenced by acids is illustrated in the following table, in which the values represent the number of cubic centimetres of a normal solution of caustic soda required to neutralise the acid liberated in a series of comparable experiments, in each of which 0·3 gramme of seed residue, 5·0 grammes of castor oil and 5 c.c. acid of the concentration stated at the head of the table were digested together during 20 hours.

Concentration of acid...	N/100.	N/50.	N/17.	N/10.	3N/10.	N/2.
Acetic....................	5·45	14·9	15·4	14·6	14·6	13·6
Succinic	2·8	14·6	15·5	15·4	14·3	12·2
Citric	7·25	15·3	15·2	14·7	7·3	1·1
Tartaric	6·95	15·4	14·5	14·2	2·0	

That the strength of the acid is a factor in the action can scarcely be doubted when the above values are contrasted with the values deduced from the electrical conductivity given by Kohlrausch:—

Acetic acid .... $K = 0·0018$
Succinic „ .... 0·006
Citric „ .... 0·082
Tartaric „ .... 0·097

It is easy to understand that the acids produced when natural fats and oils are hydrolysed have little or no influence on the enzyme, as they are not only weak acids, but also very slightly soluble in water; the influence of the acid therefore is soon at a maximum.

Dr. Nicloux, in drawing attention to his work on Ricinus Lipase,* has stated that the cytoplasm separated from castor oil seed "acts on fat in the same way as an enzyme and follows all the laws of enzyme action"; yet he concludes, that, "Nevertheless the active substance of which cytoplasm is but probably the support is not an enzyme; this substance"—which he proposes to call lipaseidine—"is destroyed by water as soon as it is no longer protected by fats."

We cannot help thinking that this conclusion is illogical and that the destruction of the lipase prepared by Nicloux is probably to be traced to an admixed proteoclastic enzyme rather than to water. The substance in question was obtained by expressing the oil from the seed, then centrifugalising the oil to separate the suspended solid matter and washing this latter free from oil with carbon bisulphide. It was intensely active as a lipoclast.

The material we have used, though free from oil, is but slightly affected by water or dilute acids. After such treatment, however, it loses to a considerable extent the property of causing oil to form an emulsion with water;

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* *Ibid.*, B, 1906, vol. 77, p. 454.

we are inclined to attribute its inferior activity principally to this effect. Our results are as follows:—

0·3 gramme of seed residue was digested with 3 c.c. of water during the time stated, 5 grammes of castor oil and 3 c.c. N/5 acetic acid were then added; the mixture, having been well shaken, was then maintained during 21 hours at 25°. The figures represent the number of cubic centimetres of N/NaOH required to neutralise the liberated acid.

Time 0 mins.	13·6
10 „	12·1
30 „	12·4
60 „	10·6
120 „	10·9

0·3 gramme of seed residue was digested with 3 c.c. N/5 acetic acid during the time stated, 5 grammes seed residue and 3 c.c. water were added, the mixture well shaken and then maintained during 19 hours at 25°; the number of cubic centimetres of N/NaOH required to neutralise the acid liberated at the end of this period was as follows:—

Time 0 mins.	15·8
10 „	14·5
30 „	15·3
60 „	10·9
120 „	11·8
180 „	10·9
94 hours	12·4

A sample was allowed to remain in contact with the acetic acid during 168 hours and then shaken with it in the shaking machine during 3 hours; after 19 hours 14·65 c.c. N/NaOH was required to neutralise the acid which was liberated from 5 grammes of castor oil.

Several experiments were made in which a large excess of water was taken and the mixture shaken in the shaking machine in order to reduce to a minimum the decrease in the amount of fat hydrolysed due to the differences in the character of the emulsion. 0·3 gramme of seed residue and 0·4 c.c. of toluene was allowed to remain in contact with (*a*) 10 c.c. N/5 acetic acid and 10 c.c. water, (*b*) 20 c.c. water, during the time stated; 5 grammes castor oil was then added and to (*a*) 10 c.c. water and to (*b*) 10 c.c. N/5 acetic acid, and the mixtures were shaken during 5 hours at 15° to 17°. The number of cubic centimetres of N/NaOH required to neutralise the acid liberated was

(a)	
Time 90 hours	3·2
3 weeks	2·8

(b)	
Time 0	5·6
90 hours	2·6
3 weeks	1·7

According to Nicloux, glycerol retards the hydrolysis. Our experiments indicate that up to 25 per cent. glycerol has little, if any, influence; in larger proportion, it certainly retards hydrolysis. All observers agree that alcohol hinders the change: our experiments show that its influence is approximately proportional to the amount which is present.

The ethereal salts which are hydrolysed under the influence of lipase are all compounds of the type

$$R'.CO.OX'.$$

Since R′ and X′ may be varied within wide limits, it cannot well be supposed that the selective action of the enzyme is exercised with reference either to R′ or X′: consequently the controlling influence must be attributed to the carboxyl radicle (CO.O); the enzyme must be so constituted that it can "fit itself to this group."

The problem to be solved is—why should ethereal salts derived from the lower terms of the acetic series be so much less readily hydrolysed than the higher? The differences in stability do not account for the differences in behaviour of homologous salts; in fact, ordinary hydrolytic agents appear to act more readily on the lower terms. Nor can the difference be attributed to the destruction of the enzyme by the acid which is liberated from the salt, as this destructive effect can be avoided by diluting the solutions to the necessary extent.

Our experiments have led us to form the provisional hypothesis that the hydrolysis of the ethereal salt by lipase involves the direct association of the enzyme *with the carboxyl centre* and that such association may be prevented by the "hydration" of this centre: consequently, that those salts which are the more attractive of water will be the less readily hydrolysed. The facts generally seem to be in accordance with this view, inasmuch as the solubility in water of ethereal salts diminishes as the series is ascended; salts such as ethylic formate and acetate undoubtedly tend to form hydrates (hydrols) in solution, such as

$$CH_3.CO.OEt + OH_2 = CH_3.C(OEt)<\begin{matrix}OH\\OH\end{matrix}$$

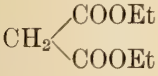
The following comparative results are of interest from this point of view:—

0·3 gramme of seed residue.
1 c.c. ethereal salt.
0·1 c.c. of toluene.
5 c.c. of N/5 acetic acid.
Water to 50 c.c.

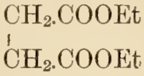
Cubic centimetres N/5 NaOH required to neutralise Acid liberated by Lipase after—

	29 hrs.	6 days.
Ethylic malonate	5·5	10·35
„ succinate	11·75	30·75
„ malate	3·5	13·5
„ tartrate...............	0·5	0·5

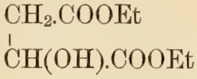
The formulæ of the four salts are as follows:—


$$CH_2\begin{cases}COOEt\\COOEt\end{cases}$$

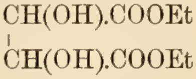
Et. malonate


$$\begin{array}{l}CH_2.COOEt\\|\\CH_2.COOEt\end{array}$$

Et. succinate


$$\begin{array}{l}CH_2.COOEt\\|\\CH(OH).COOEt\end{array}$$

Et. malate


$$\begin{array}{l}CH(OH).COOEt\\|\\CH(OH).COOEt\end{array}$$

Et. tartrate

In view of the position which it occupies between the succinate and tartrate and of its relation to ethylic malonate, the behaviour of ethylic malate is of special interest.

Other noteworthy results are the following:—

	20 hrs.	44 hrs.
Ethylic acetate	0·75	0·7
Ethylic butyrate	6·0	10·0
Amylic acetate	2·5	4·5
Ethylic malonate	5·5	8·0
Ethylic dimethylmalonate	0·75	0·75
Ethylic benzoate	0·75	1·0
Methylic salicylate	0·5	0·5
Ethylic mandelate..................	0·5	0·5
Methylic oxalate	—	—

The fact that amylic acetate, which is far less soluble in water than ethylic acetate, is more susceptible to lipase is an interesting confirmation of the explanation given above.

As it appeared possible that the differences in the rates at which different salts were hydrolysed might be due to the poisoning of the enzyme, the finely ground seed residue was mixed with the ethereal salt and the mixture shaken during an hour; the solid was then filtered off, washed with ether and dried. This material was used in the following experiments in which 0·3 gramme seed residue, 0·1 c.c. toluene, 5 grammes castor oil, 3 c.c. N/5 acetic acid and 3 c.c. water were digested together. The number of cubic centimetres of N/NaOH required to neutralise the acid liberated during 20 hours was—

Treatment of seed residue.	
Untreated	14·6
Alcohol	9·0
Ethylic butyrate	14·9
Ethylic acetate	14·65
Ethylic tartrate	11·3
Methylic sulphate	0·6

Comparison of Animal with Vegetable Lipase.—Dr. Harden has been good enough to prepare for us at the Lister Institute a quantity of animal lipase, following the directions given by Dakin.* A fresh pig's liver was minced and mixed with Kieselguhr and sand; the expressed fluid (450 c.c.)—a dark red liquid—was then centrifugalised to remove a small amount of suspended jelly. The fluid was diluted with water in various proportions for the experiments. This lipase acted readily on ethylic butyrate and to a very slight extent on ethylic tartrate. The following results were obtained, using 1 c.c. ethereal salt, 50 c.c. lipase solution and 0·2 c.c. toluene:—

Percentage of lipase solution.	Cubic centimetres of N/5 alkali corresponding to acid liberated by enzyme after 48 hours at 25°.	Percentage hydrolysed.
Ethylic butyrate—		
5	35·2	90
2½	26·95	69
1	20·9	53
½	17·45	44
2½ + 10 N/5 acetic acid	20·05	51
Ethylic tartrate—		gramme.
5	2·5	= 0·0515
5 + 10 N/5 acetic acid	1·75	= 0·036

The action of the two lipases was contrasted in a series of experiments using mixtures (*a*) of 1 c.c. of ethereal salt, 0·2 c.c. toluene and 50 c.c. of 2-per-cent. lipase solution, (*b*) of 1 c.c. ethereal salt, 5 c.c. N/5 acetic acid, 0·2 c.c. toluene, 0·5 gramme castor oil seed residue, water to 50 c.c.

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* 'Journal of Physiology,' vol. 32, p. 202.

The results show that the difference is probably only one of degree and also illustrate the comparative instability of the animal product.

	Cubic centimetres of N/5 alkali corresponding to acid liberated by enzyme at 25° after—			
	Animal.		Vegetable.	
	20 hours.	68 hours.	20 hours.	68 hours.
Ethylic malonate	9·65	10·35	3·5	7·5
Ethylic dimethylmalonate	4·0	4·35	none	none
Ethylic succinate	22·15	24·9	15·5	25·8
Ethylic malate	7·65	7·85	2·25	6·0
Ethylic tartrate	none	0·3	none	none

From the following results it seems that if only sufficient enzyme be used all ethereal salts are more or less attacked:—

Animal lipase.	Vegetable lipase.
1 c.c. salt + 0·2 c.c. toluene + 20 c.c. 2 per cent. liver juice.	1 c.c. salt + 20 c.c. water + 2 c.c. N/5 acetic acid + 0·2 c.c. toluene + 1·0 gramme seed residue.

	Cubic centimetres of N/5 alkali corresponding to acid liberated by enzyme at 25° after—			
	Animal.		Vegetable.	
	21 hours.	68 hours.	21 hours.	68 hours.
Ethylic mandelate............	1·4	1·4	1·9	2·0
Ethylic benzoate	2·8	3·9	0·68	0·8
Methylic salicylate	0·3	0·3	0·2	0·4
Ethylic acetate	3·6	10·35	3·3	9·9
Ethylic tartrate................	0·2	—	1·0	

It is proposed to study the action of lipase very thoroughly from the point of view of the working hypothesis now brought forward. A comparative examination of the enzyme derived from various sources will also be undertaken, both in order to ascertain whether only one form of lipase exists and to obtain the hydrolyst in a really suitable form for the quantitative study of its effects. Should the explanation we have advanced be justified, ethereal salts will be a material at least as valuable as that afforded by the carbohydrates for the comparative study of enzymes and acids as hydrolytic agents.

Appendix. (August 14, 1906.)

Since the foregoing account was written, a large number of experiments have been made with the object of contrasting animal lipase with vegetable lipase. We have been led gradually to recognise that in the case of the former especially, if an effective comparison is to be made between ethereal salts, it is an essential condition of success that the substances compared be in solution. We were led to this conclusion in the first place by observing in certain experiments, in which the materials were only partially dissolved, that ethylic propionate was more acted upon than was either the acetate or butyrate; but when solutions of equivalent quantities of the three salts were acted upon by liver lipase, the acetate proved to be the most and the butyrate the least stable. We need scarcely point out that this circumstance renders a strict comparison of ethereal salts which are only very slightly soluble in water a difficult matter; moreover, it must be taken into consideration in connection with our earlier experiments. Probably it is on this account also that the liquid expressed both from the liver and the pancreas acts so slowly and to so slight an extent on natural fats and oils. With these agents, however, it appears to be impossible to secure anything approaching to the complete emulsification of fats which is readily effected by the residue of castor oil seeds; indeed, at present we are inclined to attribute the extraordinary activity of the seed residue to its emulsifying power rather than to any inherent superiority of the enzyme as a lipoclast; but it should be mentioned that any treatment which renders the enzyme in the seed residue inactive also destroys the emulsifying power of the material.* All attempts which we have made to overcome the difficulty referred to by dissolving the fat or oil in neutral liquids such as toluene or ether and then violently agitating the solution with liver juice have proved unsuccessful—such treatment having served only to destroy the enzyme.

In confirmation of the statement already made with reference to the remarkable and, as we believe, significant difference in behaviour of the allied salts, ethylic succinate, malate and tartrate, the following results may be quoted which were obtained by digesting solutions of equivalent quantities of the three compounds with liver lipase. The figures represent the quantity of alkali required to neutralise the liberated acid; at the end of the experiment the succinate was practically all hydrolysed:—

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* It is not improbable that the increased activity of pancreatic juice (from a Pawlow fistula) in presence of bile salts, to which Magnus has recently called attention ('Zeits. Physiol. Chem.,' 1906, vol. 48, p. 376), is due to the promotion of emulsification by the salt.

	1 hour.	3 hours.	24 hours.
Ethylic tartrate	0·9	1·2	1·8
„ malate	2·70	5·7	15·0
„ succinate	13·50	14·5	20·4

We have to thank Mr. R. R. Armstrong, B.A., for valuable assistance rendered in the latter part of the inquiry.

The Action of Plants on a Photographic Plate in the Dark.

By W. J. Russell, Ph.D., F.R.S.

(Received June 19,—Read June 28, 1906.)

[Plates 19–21.]

It has been shown in former papers that wood has the property of acting in the dark on a photographic plate, when placed in contact or in proximity to it. Not only does wood act in this way, but leaves, seeds, roots, bulbs, and, in fact, with only few exceptions, all vegetable substances act in a similar way. The more important bodies which are without this property are starch, cellulose, gum, sugar, pith, and pollen. To obtain this action on a plate it is necessary that the body used be tolerably dry, or else the moisture contained in it will act on the gelatine of the photographic plate and destroy the picture. The time necessary for the exposure to the plate varies from a few minutes to 18 hours or more. To quicken the action, heat may be applied, but the temperature must not be above 55° C., nor the time of exposure, under ordinary circumstances, longer than 18 hours, or the photographic film will be injured. Any ordinary rapid photographic plate may be used, and its development is exactly the same as that of an ordinary picture. The best and most general method of drying vegetable substances before exposing them to the photographic plate is to place them between pure white blotting paper and subject them to considerable pressure, say from 1 to 5 or 6 tons per square inch. This process has also the advantage of giving a second picture, for it is found that the liquid which has been expressed and absorbed by the blotting paper is capable of acting on a photographic plate, and that it gives a good representation of the plant from which it came (Plate 19, fig. 1, an oak leaf).

Since different woods are capable of acting on a photographic plate it was to be expected that leaves, stems, flowers would do the same. This has been found to be the case, and the action has been traced from its commencement in the sprouting embryo of a plant till after its death. Postponing for the present the full discussion of the cause of this activity of vegetable matter on a photographic plate, it may be assumed as a working hypothesis that the active substance is hydrogen peroxide. As far as the activity of a plant goes, it is comparable with that of hydrogen peroxide. For instance, if one part of pure peroxide be dissolved in one million parts of water it is sufficient to enable the solution in 24 hours at ordinary temperatures, even when the plate is $\frac{1}{8}$ inch above the liquid, to give a marked action on the plate; and, on the other hand, a seed leaf of a runner bean weighing only 0·02 gramme can give a similar effect.

Taking now the case of a bean, both the broad and the runner bean act in the same way; the cotyledons when tested by bringing them in contact with the photographic plate, either whole or in a crushed state, are always found to be quite inactive; the plumule and radicle before growth has begun is also without action, and even when growth has just commenced and the plumule lengthened 2 or 3 mm. it is still apparently unable to act on a plate, but as soon as plumule and radicle have increased in length about 15 to 20 mm., then it has become distinctly active. The following experiments illustrate this point:—

A plumule and radicle 8 mm. long, taken from a dry bean, was entirely inactive. Another bean which had been in moist sand for four days, and was 13 mm. long, and just beginning to grow, was still unable to act on a photographic plate, but in another case in which the plumule and radicle had grown to the length of 19 mm. there was a faint action on the plate, and where the growth had extended to 80 mm. the liquid from this plant was distinctly active, and lastly, a young bean plant, 170 mm. long, contained a liquid which was very active. The cotyledons remain always quite inactive. Wheat in its ordinary dry condition is without action on a plate, but if allowed to sprout by being placed in moist sand for about two days the shoots on being squeezed between paper show considerable activity. In fact, it appears that all seedlings contain in every part, plumule, radicle, and seed leaves, a juice which is capable of acting on a photographic plate. If seedlings be grown in the dark they still have this power.

In addition to the bean and wheat, other seeds and fruits have been experimented with, specially acorn, horse chestnut, pea, barley, almond, and many others, and the conclusion is that in their embryo state they are devoid of all power of acting on a photographic plate, but that as soon as growth commences this power appears.

Supposing, as has more than once been suggested and is strongly urged by

Usher and Priestley,* that formaldehyde and hydrogen peroxide are the first products of growth, this action on a photographic plate is exactly what would occur. At the same time it must be remembered that terpenes and resins, which are so commonly present in plants, can even in very small quantities, either directly or indirectly, give rise to this body.

Nuts such as walnut, Brazil nut, Barcelona nut, etc., act like seeds; the endosperm is inactive in its original state, but if charged, as nuts often are, with a considerable amount of oil, this, on exposure to the air, oxidises and becomes very active. For instance, if the kernel of a Brazil, Barcelona, walnut, or almond nut be taken out of its shell and at once crushed between blotting paper, neither nut nor expressed liquid are active, but if the paper with the expressed liquid in it be kept for a short time exposed to the air then it gives a very dark picture. If the nut be extracted with ether and the solution evaporated, the residue becomes strongly active. Of all nuts and seeds examined, castor-oil seed appears to be by far the least active; the expressed liquid from it may be exposed for a month or more, and it is still without the power of acting on a plate.

The examination of different bulbs has shown that the fleshy scales forming them, from centre to outside, are active, but that the nucleus of the future plant which it contains, if in its primary state, is inactive like the plumule in a seed, but when growth has commenced it is active. This is well seen in the snowdrop, onion, and shallot; the shoot, if not visibly grown, is quite inactive. The amount of activity of different bulbs varies very considerably: the snowdrop bulb is slightly active, whereas the narcissus and the tulip bulb is much more so. This variation may probably depend to a considerable extent upon the state of development of the bulb. In the case of the crocus, which has a solid bulb, its activity is like those above mentioned. The tuber of the potato squeezed between blotting paper gives a very active juice, the Jerusalem artichoke a juice which is much less active. If bulbs or tubers be dried even at ordinary temperatures they lose their activity. The rhizome in different plants varies much in its activity, and probably in the same plant at different times of the year; in the iris it is but very slightly active, in sea kale it is more so, and in the Pteris it is very active.

A considerable number of different roots have been experimented with, and in all cases it has been found that the root has very considerable activity; in fact, the root of some of the larger trees—for instance, Scotch fir—gives a picture similar to that given by the wood itself.

There are some interesting points with regard to the shells which protect different seeds and fruits. The outside is quite inactive; even if laid on a plate for a long time it produces no effect. The horse chestnut is apparently an exception, but in this case the outside skin of the nut is very thin, and the brown material immediately beneath it is very active, but the nut itself, as before stated, is quite inactive.

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* 'Roy. Soc. Proc.,' B, vol. 77, p. 369.

With regard to the woody substance which forms the substance of a shell it consists of at least two distinct substances, one of which is able to act on a plate, and the other is not able to do so.

For instance, in a cocoanut shell, the constituent of the shell which is lightest in colour is quite inactive, and the darker part is very active. Again, in the butter nut, fig. 2, the red portion of the shell is quite inactive, while the dark part is strongly active. In the Brazil nut the same thing also occurs, the central dark layer being very active, and the outside part is entirely without action. In fir cones the same kind of thing occurs; the axis of a cone consists of materials varying but slightly in colour, but very considerably in their power of acting on a plate. In the case of the almond shell it is throughout perfectly inactive.

Both the shell of the acorn and of the Barcelona nut consists of two layers, which are easily separated; the outside one is inactive and the inside one is active. Immediately within the shell of a nut there are one or more integuments, and they have very different powers of acting on a plate. For instance, in the cocoanut there are three integuments, the one next to the shell is very thin and of a whitish colour, the next one is much thicker and of a brown colour, and the third one is very thin and also brown in colour. The thin integument next the shell and the thick brown one in contact with it are both active, but the third layer is without activity. There is also a thin skin adhering to the kernel; this is also quite inactive. This variation in the action of different parts of a nut is well shown in fig. 3, which represents the two halves of a walnut split through the middle; from one half the kernel has been removed, but not from the other half; the kernel filling up the shell is quite inactive, but the lining to the empty shell is very active.

Actions of the same kind occur with broad and runner beans and other seeds; the outside, the testa, is inactive and the inner layer is active.

Fig. 4, Plate 19, is a picture given by a section of a broad and a runner bean, and fig. 5 a section of a horse chestnut.

On examining the flowers of different plants the petals in all cases have been found to be active. They are best dried by squeezing between Ford blotting paper, and a picture can be obtained both from the petal itself and from the expressed juice in the paper. In many cases a characteristic picture is obtained from the paper. Fig. 6, Plate 20, is one obtained from the paper on which an Auricula flower has been squeezed, and fig. 7 is from an Oriental poppy. Fig. 8 is from the petal of a viola. The colour of the petal does not affect the result. White, blue, and pink petals of the Canterbury bell, red and white rose petals, dark purple and yellow viola petals and many others all give similar results, and all, as far as rough experiment goes, appear to be active to about the same extent. They show, however, greater activity than an equal area of the leaf of the same plant.

The pistil in a number of plants, for instance, poppy, lily, cactus, tulip, iris, etc., were examined, and in all cases were found to be very active. The stamens of different plants when squeezed were found to be active, but pollen, on the contrary, when examined alone was always found to be quite inactive. That from the sedge, hazel, Scotch fir, *Pinus pinaster*, *Pinus cembra*, and many other plants was examined.

Leaves.—Carrying the investigation on to leaves, large numbers have been experimented with, and all have been found to act on a photographic plate. The amount of action naturally varies with leaves from different plants; where there is much sap juice, there is generally much action. If a leaf be simply dried its activity is much lessened, or may be nearly destroyed, and its surface is rendered uneven; but even in old and dried leaves a very appreciable amount of activity remains. For instance, beech leaves picked up off the ground were found to have about the same degree of activity as the ordinary green leaf, and a canna leaf which had been between blotting paper in a press for one and a-half years, and other leaves which had been pressed for two to three years, still were able to give a faint picture. If these old leaves were slightly moistened before putting up with a photographic plate, this activity was considerably increased (figs. 9 and 10). Also it is interesting to note that if a slit be cut in the dry leaf, this becomes in the picture very visible. Fig. 11 appears as if an active emanation had taken place from the body of the leaf. The best way of obtaining satisfactory pictures of leaves is to subject them to considerable pressure between white (Ford) blotting paper; this, of course, squeezes the leaf juice out and the leaf is left in such a condition that after standing in the air for a short time it may be put up with the photographic plate. Whether the squeezing should be repeated, and whether the pressure should be a quarter of a ton or six tons, depends on the nature of the leaf experimented with. This way of drying a leaf has also the advantage, as previously pointed out, of giving a second picture, which is often a very interesting one, and is simply obtained by exposing the paper with the absorbed sap from the leaf to a photographic plate.

Figs. 12 and 13, Plate 21, are from the leaf itself, black currant, and figs. 14 and 15 are pictures of the same leaf, but are from the paper between which it was squeezed. As further illustrating the nature of the pictures obtainable by these processes, fig. 16 shows the front and back of a beech leaf taken direct from the leaf. Ferns and mosses also give interesting results, but these will not be dealt with in the present communication.

The above investigation has been carried out in the Davy-Faraday laboratory, and my thanks are due to the managers of the Royal Institution for the use of their laboratory. I am also much indebted to my assistant, Mr. O. F. Bloch, who has been of much help to me, and has made all the photographic pictures for the illustrations.

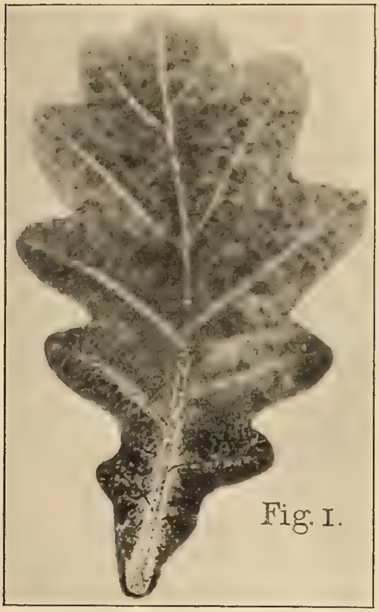

Fig. 1.

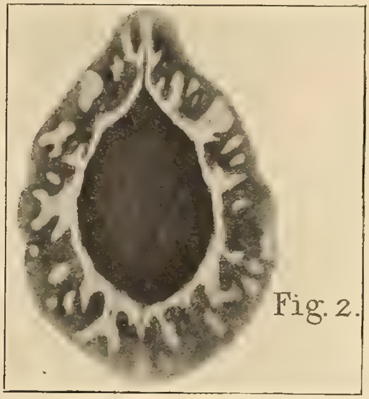

Fig. 2.

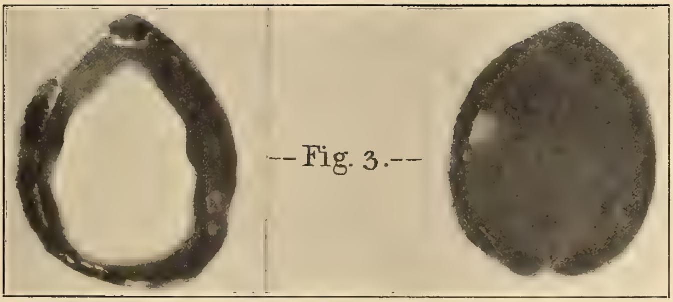

Fig. 3.

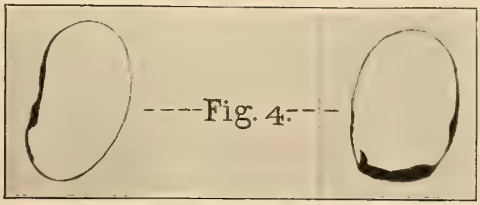

Fig. 4.

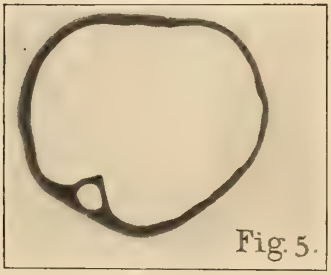

Fig. 5.

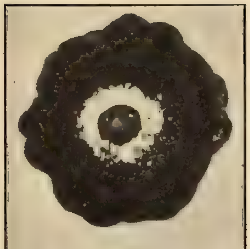

Fig. 6.

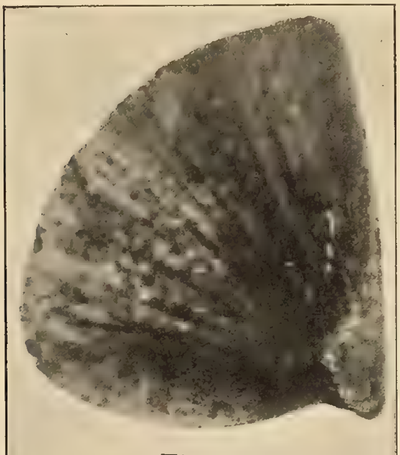

Fig. 7.

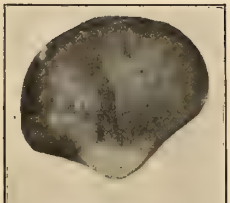

Fig. 8.

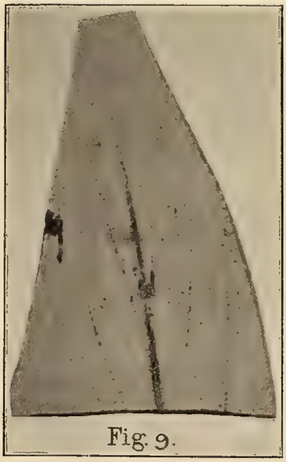

Fig. 9.

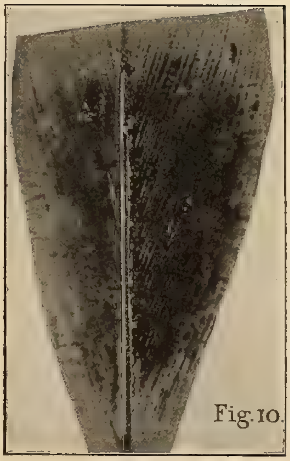

Fig. 10.

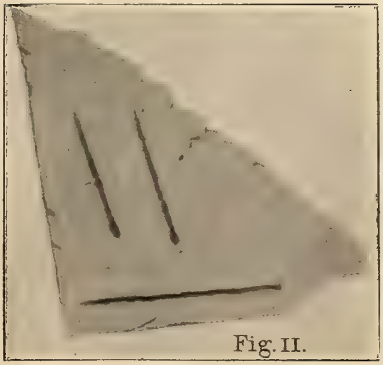

Fig. 11.

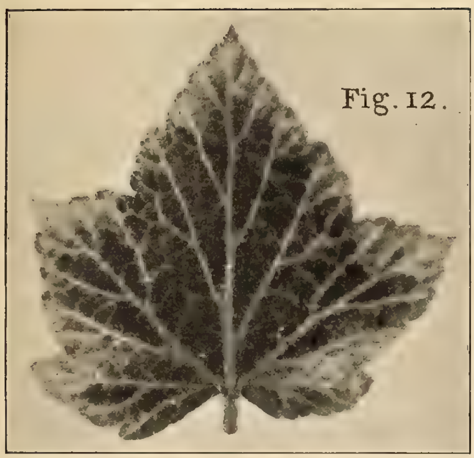
Fig. 12.

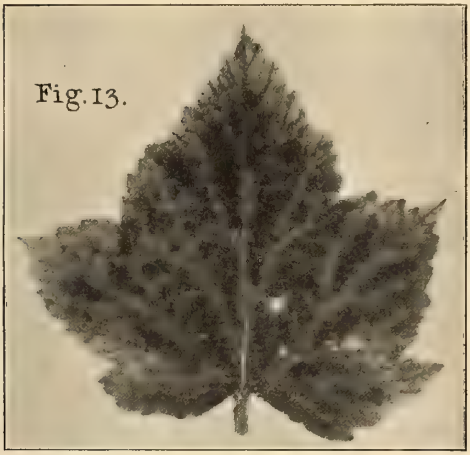
Fig. 13.

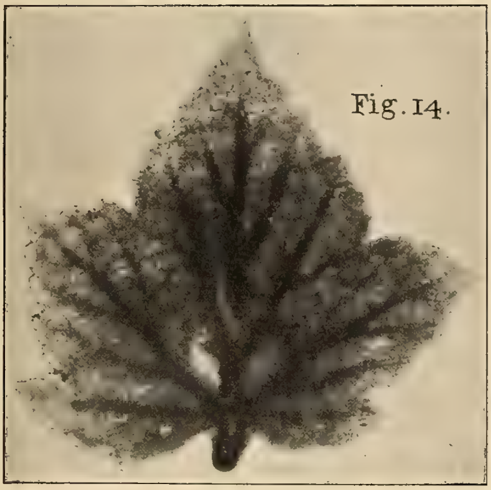
Fig. 14.

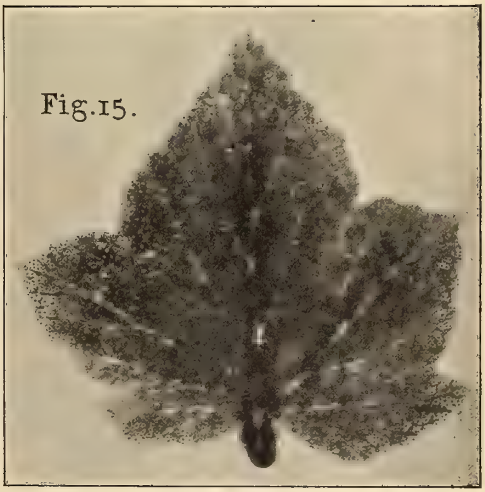
Fig. 15.

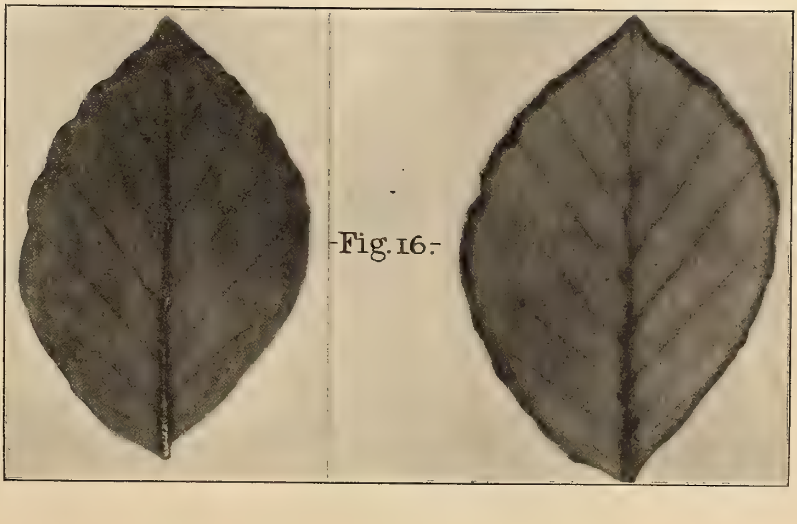
Fig. 16.

The Pharmacology of Ethyl Chloride.

By E. H. Embley, M.D., Hon. Anæsthetist to the Melbourne Hospital.

(Communicated by Dr. C. J. Martin, F.R.S. Received June 13,—Read June 28, 1906.)

(From the Physiological Laboratory of the University of Melbourne.)

The rapidity with which ethyl chloride has established its claims as a general anæsthetic for short operations appears justified by the advantages which its employment offers. It induces anæsthesia pleasantly and very rapidly, and compares favourably with nitrous oxide in these respects. The rapidity with which anæsthesia is induced, and the comparatively prompt recovery upon cessation of the administration, combined with the relatively small degree of vomiting or nausea, are its great advantages over ether and chloroform. The greater duration of anæsthesia following upon cessation of administration together with its portability are its advantages over nitrous oxide. Its position, therefore, in the armamentarium of the anæsthetist is that of filling the important gap between the light and brief anæsthesia of nitrous oxide and the deep narcosis of chloroform or ether.

This position is held, however, not without disabilities. In the first place muscular relaxation is not often completely attained until a deep degree of narcosis is reached and, more important by far, it is not as safe as nitrous oxide. The death rate is much greater than that of nitrous oxide and according to McCardie (1), is as high as 1 in 3000.

Ethyl chloride was first used as a general anæsthetic in 1848 by Heyfelder. Benjamin Ward Richardson experimented with it in 1867 and it was the subject of an enquiry by a Committee of the British Medical Association in 1880, who reported unfavourably upon its employment as a general anæsthetic. The revival of ethyl chloride as an anæsthetic for short periods occurred in 1895—6 and an historical account of its re-introduction will be found in a paper, "Ethyl Chloride as a General Anæsthetic," by W. J. McCardie (2). According to McCardie some experimental observations upon its pharmacological action have been made by Wood and Cerna, Koenig, Malherbes, and Roubinovitch, but I regret that the literature has not been available, and I have been unable to consult these papers.

The physiological action of a commercial preparation containing ethyl chloride 65 per cent., methyl chloride 30 per cent., and ethyl bromide

5 per cent., sold under the name of "Somnoform," was investigated by Cole (3).

Cole's experiments were performed for the most part upon rabbits, but in three cases cats were used. The quantity of Somnoform in the air inspired was not ascertained. Cole found the effect upon the respiration to be at first stimulative, followed, when large doses were employed, by standstill of the respiration, with the diaphragm in a condition of tonic contraction. The changes in the circulation were increased rate and diminished power of cardiac contractions and gradual fall of blood-pressure. No effect upon the vaso-motor system was discovered. The vagus endings in the heart were paralysed.

Interesting clinical observations have been reported by McCardie (1), Hewitt (4), and others.

McCardie's papers are particularly valuable, as, besides detailing the results of an extensive personal experience with this anæsthetic, he has collected as far as possible the experience of others at home and on the Continent.

The present paper records the results of work undertaken with a view to studying the pharmacology of ethyl chloride, in the hope that this might be of some value to those who may be concerned with its administration, and with the view of saving what appears to me to be a valuable anæsthetic agent from coming under the suspicion of being unduly dangerous.

For better comparison with the effects of chloroform, which have been frequently studied, dogs were used in all of the experiments, since these animals have been generally employed in chloroform research. In all experiments morphine and ether were used as the preliminary anæsthetics.

THE SOLUBILITY OF ETHYL CHLORIDE IN WATER AND BLOOD.

Ethyl chloride vapour I find to be soluble in water to the extent of 253·36 per cent. by volume (0·678 per cent. by weight) at 21° C. and at 760 mm. I have also ascertained the extent of its absorption by blood. There was considerable difficulty in determining this in consequence of the blood becoming of a tarry consistence as the absorption increased. In this condition the absorption progressed very slowly; for instance, in one experiment in which 100 c.c. of blood was employed and 496 c.c. of ethyl chloride vapour had already been absorbed, it required eight hours' contact, with continual shaking, for absorption of a further 5 c.c. In consequence of this physical difficulty, the complete absorptive power of blood for ethyl chloride was not determined. It is only possible to state that blood at 38° C. takes up an amount exceeding 500 per cent. by volume of the vapour.

The determinations were made in a special absorptiometer, which was kept at constant temperature by means of a water-bath, and in which arrangements were made for continuous shaking of the bulb containing the water or blood respectively. The bulb communicated by means of a narrow-bored tube with a gas burette containing the vapour of ethyl chloride. Measured quantities of vapour were passed into the bulb from time to time as absorption progressed, and the actual quantity taken up determined. The pressure in the absorptiometer was maintained constant by a variable mercury level.

In these determinations the blood was found to lake in the earlier part of the absorption process, but no massive formation of hæmoglobin crystals occurred as in the case of chloroform under like conditions. Blood absorbs more than twice as much of the gas as water under similar conditions. Ethyl chloride, like chloroform, evidently enters into chemical union with the blood.

EFFECT OF ETHYL CHLORIDE UPON THE HEART ISOLATED FROM THE CENTRAL NERVOUS SYSTEM.

In a previous investigation (5) of the effect of chloroform upon the isolated heart the method of Hering was employed. As, however, some difficulty is apt to happen in this method in consequence of the tendency to the formation of clots in the U-cannula when the blood-pressure falls, I have devised the following method, by means of which a circuit is maintained without the introduction of foreign bodies, such as cannulæ, for establishing continuity.

It consists in the ligation of all of the arterial trunks except the left subclavian, the aorta being tied just beyond the left subclavian artery. The ascending branches of the left subclavian and the vertebral arteries are then tied. One of the carotid arteries was used for recording the blood-pressure. The vagi were divided. The blood supply to the central nervous system was thus cut out and the operation of the central nervous system was in this way abolished. The heart circulated normal arterial blood.

In experiments under the above conditions, in which simultaneous blood-pressure curves and plethysmographic records of the changes in the volume of the left leg were taken under chloroform narcosis, it was found that the changes in the blood-pressure were accompanied by corresponding changes in the volume of the foreleg (left) in which the circulation was affected.

Definite mixtures of ethyl chloride and air were administered by artificial respiration from a gasometer in which the mixture was contained. The Cambridge Scientific Instrument Company's apparatus was used for artificial respiration.

The experiments, records of which appear in figs. 1, 2, 3, and 4, demonstrate the gradually-increasing effects upon the isolated heart of varying percentages of ethyl chloride in the air inspired.

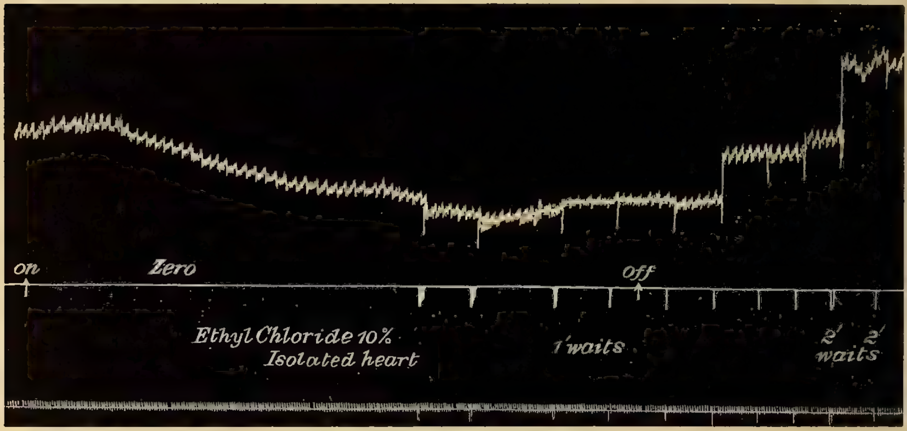

Fig. 1.—¾ size of original.

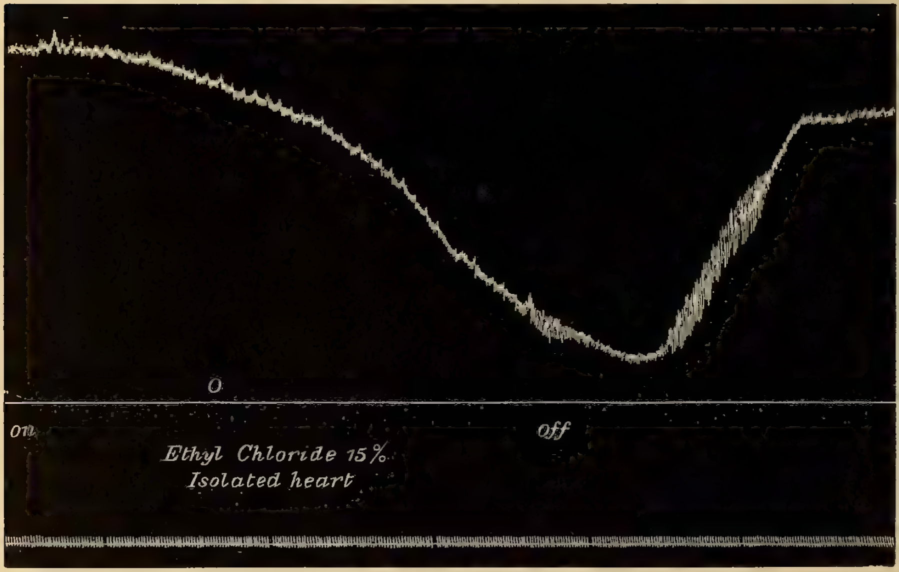

Fig. 2.—¾ size of original.

The effect upon the work done by the heart was as follows :—

Expt.	1.	10	p.c. ethyl chloride	administered.	Blood-press.	fell from	92 to 58	mm. Hg in	8′ 15″
,,	2.	15	,,	,,	,,	,,	158 ,, 63	,,	3′ 12″
,,	3.	20	,,	,,	,,	,,	160 ,, 72	,,	1′ 1″
,,		20	,,	,,	,,	,,	160 ,, 62	,,	0′ 59″
,,	4.	30	,,	,,	,,	,,	164 ,, 34	,,	0′ 46″

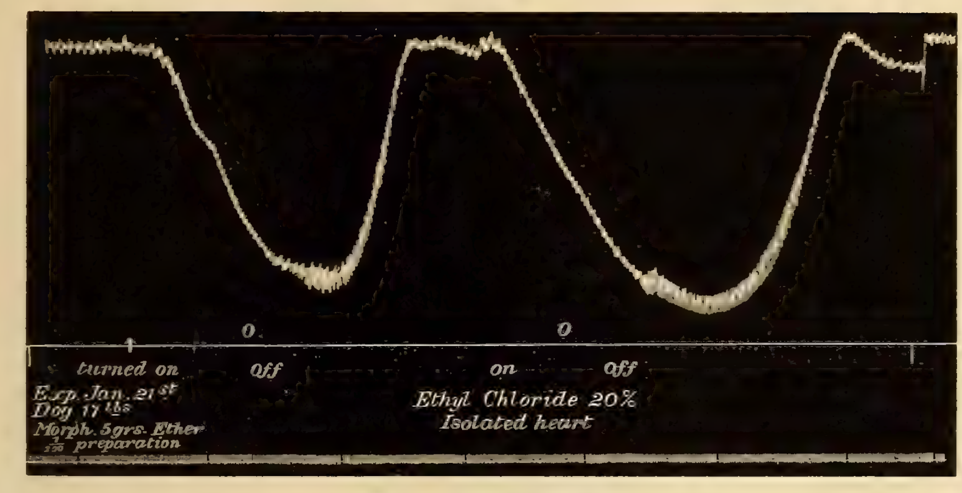

FIG. 3.—$\frac{3}{4}$ size of original.

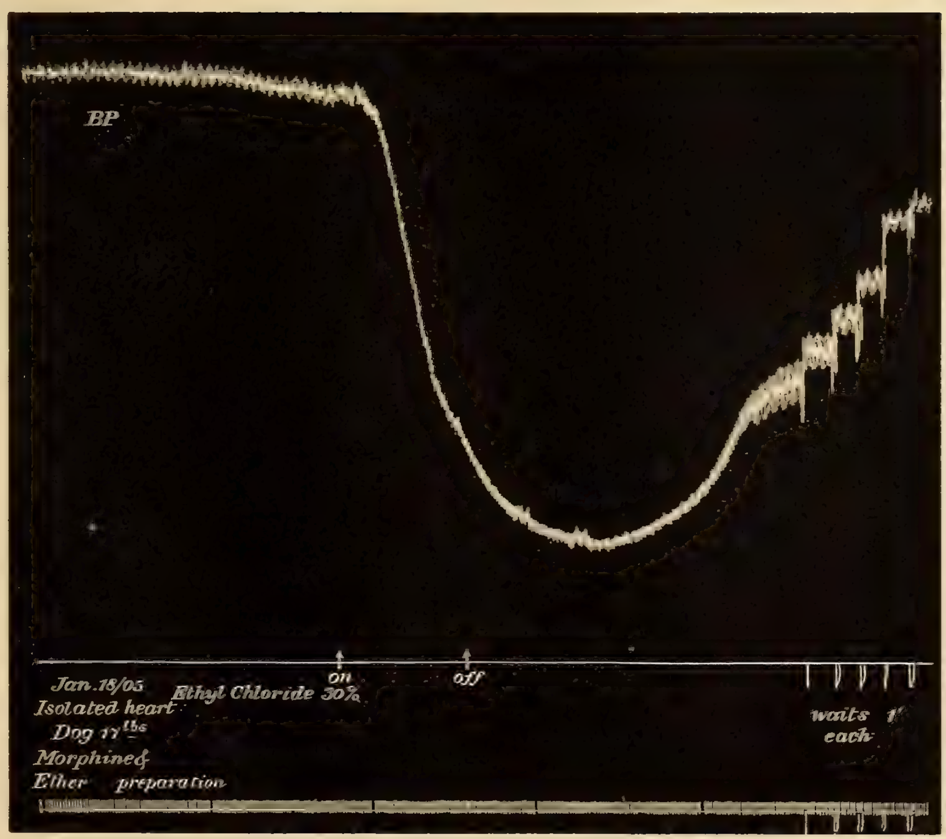

FIG. 4.—$\frac{12}{13}$ size of original.

The heart rate increased in some experiments, but it will be noticed, on reference to the tracings, that in the experiments with stronger mixtures the rate slowed after the administration had been discontinued.

In subsequent experiments on the intact animal, with natural respiration, it appeared impossible to introduce sufficient ethyl chloride into the circulation by inhalation to produce the above rapid paralysis of heart muscle, as cessation of respiration or vagus inhibition of the heart occurred to check further intake.

Conclusions.—The effect of ethyl chloride upon heart muscle, as in the case with chloroform and in contrast with that of ether, is paralytic, but the quantity of ethyl chloride vapour in the air required is 19 times as great as that of chloroform to produce comparable results.

EFFECT OF ETHYL CHLORIDE UPON THE VASCULAR SYSTEM.

This investigation was approached in three ways:—

(1) By investigating the effects of ethyl chloride upon the arterioles independent of the central vaso-motor system.

(2) By investigating its effect upon the central vaso-motor mechanism.

(3) By investigating the resultant of its combined action on the vessels and on the vaso-motor system in the intact animal.

(1) *The Effect of Ethyl Chloride upon the Arterioles.*

This was accomplished by measuring the changes in the outflow, under constant pressure, of blood circulating through a loop of excised bowel together with the isolated lungs of the animal. The method employed was that described, in a similar research in connection with chloroform, by Embley and Martin (6).

After sufficient time had been allowed for the circulatory flow to become constant, air containing 30 per cent. of ethyl chloride vapour was pumped into the lungs. The curve, fig. 5, was plotted from the tracing which recorded the drop-rate, the time, and the pressure. The ordinates represent the number of drops per minute against time in minutes along the abscissæ. The 0 and 8 perpendicular lines indicate the time during which the anæsthetic was administered. The pressure at which the blood circulated was 80 mm. Hg throughout.

The ethyl chloride was administered during 8 minutes, and it will be seen that, following upon the introduction of the ethyl chloride into the respired air, a gradual increase in flow occurred which continued after the administration had ceased. The maximum effect was a 50·5 per cent. increase. This rate

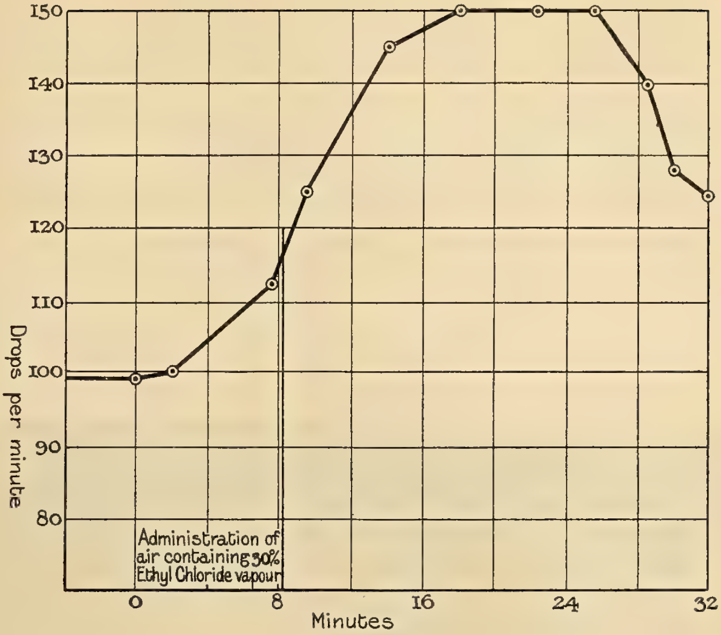

FIG. 5.

of increase was maintained for 7′ 30″, and it was 17′ 30″ after the discontinuance of the ethyl chloride ere it began to diminish.

These experiments demonstrate that the effect of ethyl chloride upon the arterioles isolated from the central nervous system is relaxation. In this respect it is similar to chloroform, but the amount required is vastly greater.

(2) *The Effects of Ethyl Chloride upon the Central Vaso-motor System.*

This was investigated by conveying the ethyl chloride to the brain alone, upon the lines pursued by Gaskell and Shore (7) in their work on chloroform. They employed an extensive proceeding, which consisted in connecting the vessels to and from the brain of one dog with the circulatory system of another and larger dog, so that the larger dog supplied the brain of the other dog. The chloroform was then administered to the larger dog and records taken of each. The brain alone of the smaller dog received chloroform. In the following experiments an artificial arterial circulation replaced that of the large dog of Gaskell and Shore's experiments.

The defibrinated blood of another dog, containing various known percentages of ethyl chloride, was delivered by the artificial arterial circulation apparatus to the brain through the two carotid arteries, for short periods at a time. The temperature of the blood in this artificial supply was 38° C., and the pressure the same as that in the femoral artery of the dog at the commencement of the experiment. The animal was curarised. Simultaneous records of arterial pressure and bowel volume were taken.

Fig. 6, showing bowel volume (B.V.) and blood-pressure (B.P.), demonstrates the effect of blood saturated with air containing 30 per cent. of ethyl chloride vapour delivered to the brain for 19, 23, and 13 seconds in the first, second, and third delivery respectively. The first effect produced is vaso-constriction as shown by the simultaneous rise in blood-pressure and diminution in bowel volume. After 4′ 15″ the second delivery was made, but the effect produced, although under the same conditions, was the very reverse of the first, for the blood-pressure fell simultaneously with the occurrence of an increase in the size of the bowel volume. The third delivery, again under the same conditions, produced effects similar to the second. These latter were obviously vaso-dilator effects.

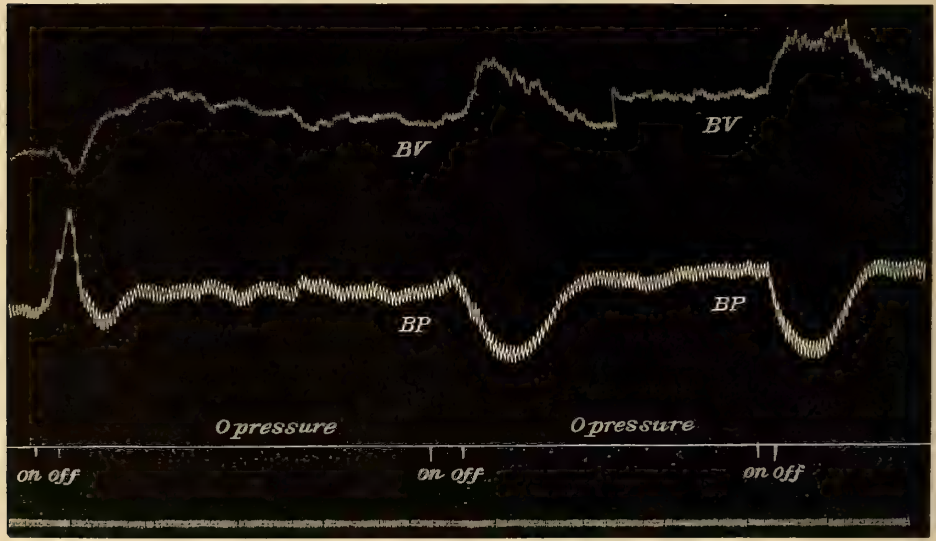

FIG. 6.—½ size of original.

Further experiments with the same dog produced combinations of these results. At one time vaso-constriction was followed by vaso-dilation in the one delivery. At another delivery vaso-constriction was followed by vaso-dilation, and then by vaso-constriction. In the last experiments the artificial circulation was continued for as long as 40″—much longer than in the former. In similar experiments upon other dogs the constrictor effects were more marked and more prolonged. When lower percentages were used the same results ensued, and the effect was roughly proportional to the concentration of ethyl chloride in the blood injected.

I conclude, therefore, that the effect of ethyl chloride upon the central vaso-motor mechanism, as was shown to be the case for chloroform by Gaskell and Shore (8) and myself (9), is, for a time at least, stimulative.

(3) *The Resultant of the Combination of the Effects of Ethyl Chloride upon Arteriolar Muscle and Vaso-motor System in the Intact Animal.*

This was investigated by taking simultaneous blood-pressure and plethysmographic records during the administration of ethyl chloride. Additional evidence was furnished by vertical rotation of animals under deep ethyl chloride narcosis.

Fig. 7 represents the simultaneous record of blood-pressure and volume of a piece of bowel, following the administration of air containing 30 per cent. of ethyl chloride to an intact dog.

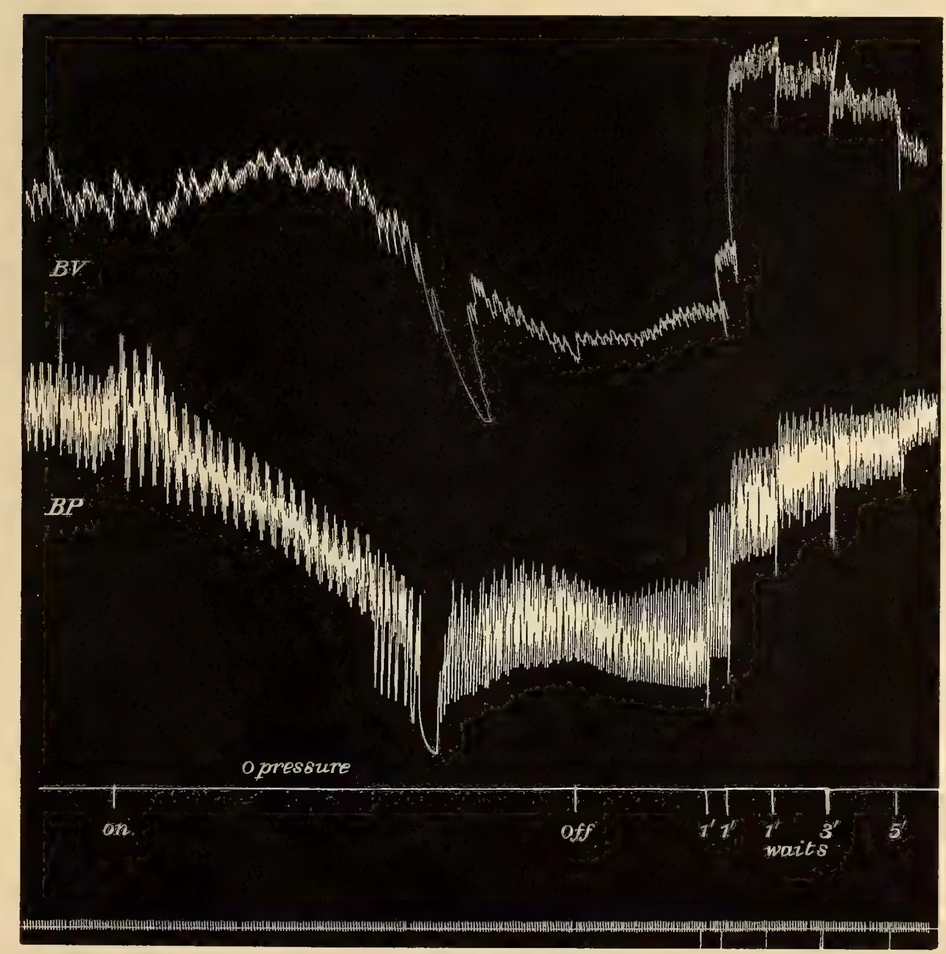

Fig. 7.—$\frac{6}{7}$ size of original.

The administration was continued for 2′ 45″. A preliminary small rise and a simultaneous small diminution in bowel volume occurred, due apparently to vaso-constrictor stimulation. This was followed by a fall in blood-pressure and increase in bowel volume, so that in 1′ 15″ the blood-pressure had fallen 46 mm. Hg, and the tambour lever of the bowel record had risen 5 mm., showing an increase in bowel volume. This increase in bowel volume, however, was not maintained against the concomitant continued fall of blood-pressure, so that the bowel volume soon diminished. The tambour lever in the ensuing 1′ 20″ fell 27 mm., whilst the blood-pressure fell a further 19 mm. After the administration had ceased and recovery had begun, a great increase in bowel volume occurred during the recovering blood-pressure, so that in 3′ 5″ after cessation of administration the lever of the tambour had risen 42 mm., which was 20 mm. above its level before starting the administration of ethyl chloride, whilst the blood-pressure had risen 39 mm. Hg—still 24 mm. below the pressure before starting the ethyl chloride. In 13′ 25″ from cessation of administration of ethyl chloride the blood-pressure and bowel volume had returned to their former levels.

This result is seen, upon comparison, to be precisely similar to those which Martin and I (10) obtained during our investigation of this question in chloroform narcosis. In each the organ volume was found to increase at first for a time, although the blood-pressure was falling, but to soon diminish as the blood-pressure continued to fall and after cessation of the administration to rise above the level that it had previous to commencing the administration, although the blood-pressure still lagged considerably below the height it had previous to the administration of ethyl chloride or chloroform. The interpretation is the same as that given in the chloroform researches above mentioned, and is that the heart recovered before the blood-vessels and poured blood into the relaxed arteries, thereby occasioning a considerably greater increase in the organ volume; and that this condition was maintained until the vessels recovered their tone, when the volume returned to its previous level.

Vertical rotation of the intact animal, with the head up, under deep ethyl chloride anæsthesia induced by artificial respiration, brought down the blood-pressure from 70 to 48 mm. Hg.

The results of vertical rotation under deep ethyl chloride narcosis are considerably less marked than those produced by chloroform. With a corresponding depth of narcosis with chloroform a fall to zero was usually observed. The paralysis of the local vaso-motor mechanism is apparently much less profound in a corresponding depth of anæsthesia with ethyl chloride.

Conclusion.—The net result of the action of ethyl chloride upon the vascular system, therefore, is dilatation, but the degree of paralysis is strikingly less than that produced by chloroform, even when the latter is present in less than one-tenth the quantity in the air inspired.

THE EFFECT OF ETHYL CHLORIDE UPON THE VAGUS MECHANISM.

This question was investigated by taking blood-pressure records of the same and different dogs with various percentages of ethyl chloride vapour in the air respired, (a) with intact vagi; and (b) after section of the vagi.

(a) *The Effect of Ethyl Chloride in varying Percentages in the Air inspired* upon the rate of the heart is seen in the table below, which is compiled from a number of experiments with different percentages of ethyl chloride in the air inhaled:—

								mm. Hg.
3·5	p.c. in	15′	caused no change in the heart rate or blood-pressure.					
7	p.c. in	13′	caused heart rate	to fall from	90 to 69	per min. and blood-press.	to fall from	127 to 80
10	„	14′ 30″	„	„	65 „ 30	„	„	195 „ 50
10	„	2′ 15″	„	„	86 „ 0	„	„	100 „ 10
15	„	6′ 10″	„	„	74 „ 0	„	„	120 „ 8
20	„	7′ 30″	„	„	90 „ 40	„	„	124 „ 90
30	„	1′ 18″	„	„	80 „ 10	„	„	120 „ 24
30	„	8′ 40″	„	„	84 „ 14	„	„	116 „ 18
30	„	3′ 20″	„	„	86 „ 0	„	„	100 „ 10
30	„	2′ 45″	„	„	90 „ 10	„	„	112 „ 50
30	„	4′ 30″	„	„	88 „ 16	„	„	104 „ 30

Figs. 8, 9, and 10 are blood-pressure curves obtained under the influence

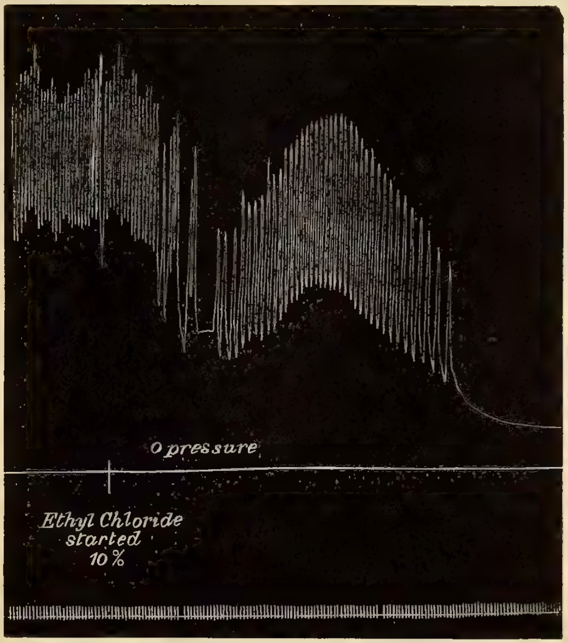

FIG. 8.—Original size.

of 10, 10, and 30 per cent. respectively of ethyl chloride in the air respired. In the above cases the vagi were intact. The varying rapidity of onset of inhibition with the same or different percentages of the anæsthetic denotes variations in the excitability of the vagus mechanism in these experiments.

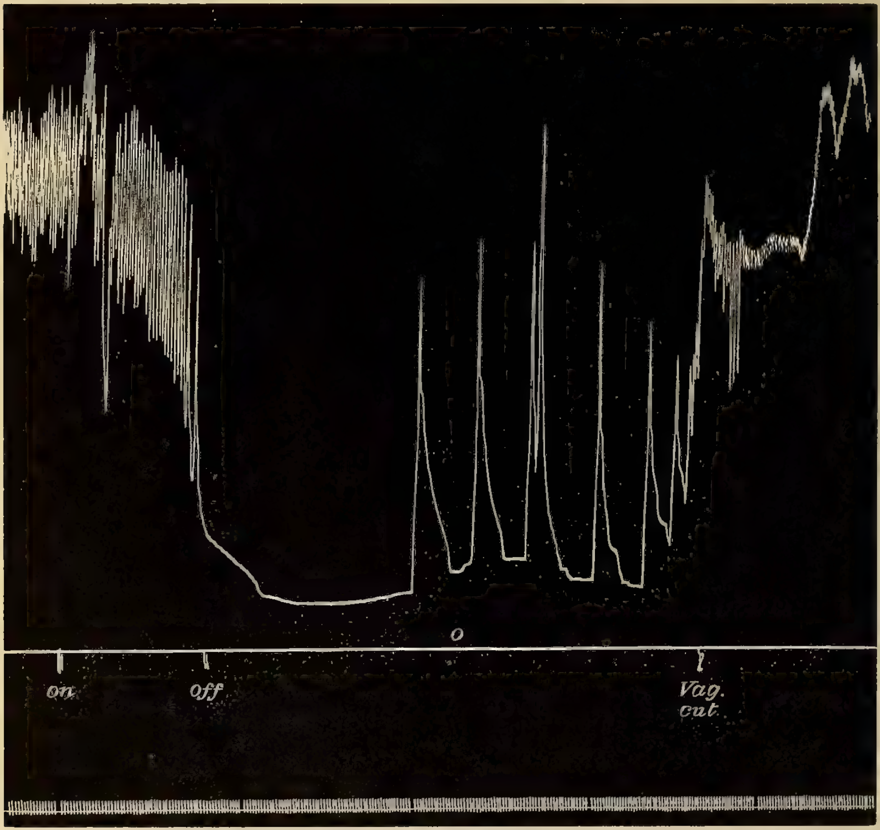

Fig. 9.—Original size.

(b) *The Effect of Ethyl Chloride in varying Percentages upon the Heart Rate after Section of the Vagi* is seen below:—

10 p.c. in 14′ caused no change in heart rate and no fall in blood-pressure.
15 „ 12 „ „ „ „
20 „ 12 „ „ „ „
20 „ 13 „ „ a fall in blood-pressure from 78 to 70 mm. Hg.
30 „ 7 „ „ „ „ „ 102 „ 38 „

The heart rate was altered in none of these experiments. In three out of the five there was no fall in pressure. In the fourth there was a fall, but it was slight. The duration of the administration in these cases was

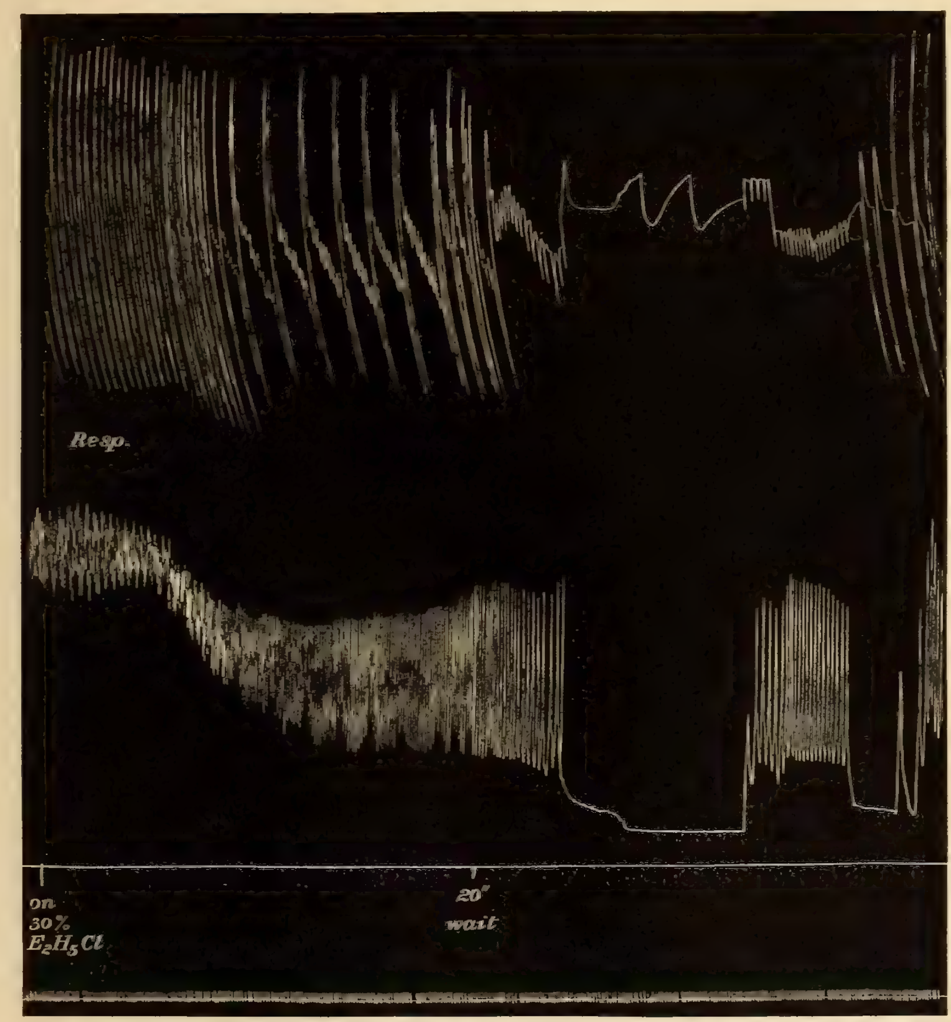

Fig. 10.—$\frac{1}{5}$ size of original.

considerably longer than that of the average (see the former table) required to bring on marked slowing of the heart rate and great fall of blood-pressure. In the fifth experiment (second table), in which 30 per cent. of ethyl chloride was administered in the inspired air, the blood-pressure fell from 102 to 38 mm. Hg in 7′ without alteration in the heart rate. In this experiment the fall of blood-pressure was due to the paralytic effect of the anæsthetic upon the heart and vessels.

From these results it is evident that great and sudden falls of blood-pressure may occur from the administration of ethyl chloride in strength of 10 per cent. and upwards, and that when the strength of the vapour does not exceed 20 per cent. in the air inspired the slowing of the heart rate from vagus inhibition is the cause of that fall. When a strength of vapour of 30 per cent. and upwards is administered the fall of blood-pressure is in part due to cardio-vascular paralysis, but a fall of blood-pressure occurring without slowing of the heart rate is due wholly to cardio-vascular paralysis.

These statements apply to dogs. They, moreover, apply for short periods—not more than 14′ periods, which, however, are longer than those during which ethyl chloride is generally used in clinical administrations.

In comparing the cases of inhibition of the heart produced by chloroform in my previous experimental work (11), I find that 2·5 per cent. chloroform vapour in the air administered produces approximately the same degree of cardiac inhibition as 10 per cent. ethyl chloride.

There is, however, a difference of vast clinical interest between the inhibition produced by chloroform and that by ethyl chloride, since inhibition due to chloroform is liable to prove fatal, whereas I have not succeeded, in this investigation, in causing a fatal case of cardiac inhibition with ethyl chloride. Furthermore, I have never succeeded in fatally arresting the heart by faradic stimulation of the peripheral ends of the divided vagi under any depth of ethyl chloride narcosis. Whereas, with chloroform, faradic stimulation under like conditions and deep degree of narcosis easily brought about this result (11).

If, however, we compare the results of chloroform (5) and of ethyl chloride upon the heart alone (p. 393), and upon the vagus mechanism alone, it is seen that whilst it requires approximately (p. 401) four times more ethyl chloride to produce the same degree of inhibition as is produced by chloroform in a given time, it requires approximately 19 (p. 396) times as much ethyl chloride to produce the same degree of cardiac depression in the same period of time as is produced by chloroform. Hence it is that in these experiments cardiac inhibition has come on early—before the spontaneous excitability of the heart has been much depressed. Herein, therefore, appears to lie the reason why 30 per cent. and upwards of ethyl chloride vapour in the air inspired has not produced fatal cardiac inhibition in the experiments with ethyl chloride. Herein, too, lies the cause of the relative safety of ethyl chloride.

That the slowing of the heart under the influence of ethyl chloride is due to vagus inhibition is proved by its prompt cessation upon section of these nerves, as is shown in fig. 9. This experiment also proves that the inhibition is not a reflex arising from stimulation of the sensory nerve ends in the mucous membrane of the nose, trachea, or bronchi, or in the alveoli of the lungs, but to direct central stimulation, for in this experiment defibrinated blood saturated with air containing 30 per cent. of ethyl chloride vapour was delivered to the brain as an independent arterial circulation, as described above (p. 397). Inhibition occurring in these experiments, in which no ethyl chloride comes into contact with the respiratory tract or the alveoli, must necessarily be central. The inhibitory action of ethyl chloride is thus similar to that of chloroform, and due to the direct action of the drug upon the vagus mechanism in the medulla.

CONCLUSIONS REGARDING THE EFFECT OF ETHYL CHLORIDE UPON THE VAGUS MECHANISM.

(1) Vagus inhibition of the heart occurs very readily when ethyl chloride vapour of a strength of 10 per cent. and upwards is administered in the air inspired.

(2) Sudden fall of blood-pressure occurring during the administration of ethyl chloride vapour in the air inspired ranging in strength from 10 per cent. to 20 per cent. is due to vagus inhibition of the heart. During the administration of 30 per cent. or upwards the fall of pressure is also due to weakening of the cardiac and arterial musculature.

(3) Cardiac inhibition is not so serious from ethyl chloride as it is from chloroform, since it comes on before the spontaneous excitability of the heart has been much depressed. It does not seem possible to permanently arrest a dog's sound heart under ethyl chloride narcosis by vagus inhibition. It requires 19 times more ethyl chloride to produce a given degree of cardiac depression than is required of chloroform, whilst it requires only four times as much to produce cardiac arrest by vagal stimulation, hence inhibition sets in relatively rapidly. Herein lies the relative safety of ethyl chloride.

(4) The cardiac inhibition arises from central stimulation. It is not reflex.

(5) No evidence of any paralysis of vagus endings, such as was described by Cole, was obtained.

INTER-DEPENDENCE OF BLOOD-PRESSURE AND RESPIRATION.

The investigation of this subject in chloroform narcosis demonstrated (12) that the integrity of the nervous mechanism of respiration was dependent upon the maintenance of blood-pressure. When the blood-pressure fell below a certain level the respiration failed and recovered when the blood-pressure rose again. In the present investigation of this subject in ethyl chloride narcosis, simultaneous tracings were taken of the blood-pressure and respiration with varying percentages of ethyl chloride vapour in the air inspired and the results compared with those of chloroform mentioned above.

The following table gives some of the results taken from 35 experiments performed in this connection.

3—5	p.c. C_2H_5Cl vapour	in air inspired.	Respiration	unaffected.
7	„	„	„	„ in 13′.
10	„	„	„	slowed in 14′.
10	„	„	„	arrested in 2′ 15″. Blood-press. at 10 mm. Hg from cardiac inhibition.
15	„	„	„	arrested in 5′ 40″. Blood-press. at 40 mm. Hg from cardiac inhibition.
18·5	„	„	„	arrested in 8′ 10″. Blood-press. at 20 mm. Hg from cardiac inhibition.
20	„	„	„	greatly slowed. Blood-press. at 68 mm. Hg from cardiac inhibition.
20	„	„	„	arrested in 5′ 35″. Blood-press. at 56 mm. Hg from cardiac inhibition.
30	„	„	„	arrested in 3′ 10″. Blood-press. at 15 mm. Hg from cardiac inhibition.
30	„	„	„	greatly slowed in 8′ 40″. Blood-press. at 18 mm. Hg from cardiac inhibition.
30	„	„	„	arrested in 3′ 14″. Blood-press. at 35·5 mm. Hg from cardiac inhibition.
30	„	„	„	arrested in 2′ 45″. Blood-press. at 50 mm. Hg from cardiac inhibition.

In each of these experiments the respiration returned as the blood-pressure recovered.

It was found, as this table shows, that the blood-pressure falls considerably before the respiration stops, which latter recovers when the blood-pressure rises again, as in fig. 10. The correspondence with the effects of chloroform is still closer, since respiration may be paralysed by ethyl chloride independently of fall of blood-pressure, but not under the ordinary conditions of administration by respiration. It occurs, for instance, when, the liquid ethyl chloride is sprayed into the pharynx, but then only when the vagi are cut or in a state of depressed excitability, otherwise inhibition of the heart would occur and a fall of blood-pressure ensue in consequence.

There is, however, a type of respiration which seems peculiar to a certain depth of ethyl chloride narcosis. It is found to occur as an antecedent to respiratory failure. It is characterised by a remarkable prolongation of the respiratory pause. The tracing closely resembles that which occurs after division of the vagi in an otherwise normal dog, but with the difference that in this case the vagi are unsevered, as is seen in the blood-pressure tracing, fig. 11, in which the successive respirations occur at the apex of each curve. The blood-pressure falls after each small group of inspirations in consequence of the slowing of the heart rate itself, a result of vagus inhibition. The further accession of ethyl chloride to the blood stream being prevented by the arrest of further respiratory intake, stimulation of the vagus mechanism passes off, the heart's rate increases and the blood-pressure rises to its former level. Another set of respirations is then taken and the same cycle of events follows. In this manner respiratory arrest is delayed. The animal is, however, becoming asphyxiated, so that either the heart must be arrested by the consequent exalted irritability of the vagi or the respiration cease. One of these occurrences is found by experiment to occur. This form of respiration does not always precede respiratory failure. It occurs generally when excessively large percentages of ethyl chloride are administered in the air.

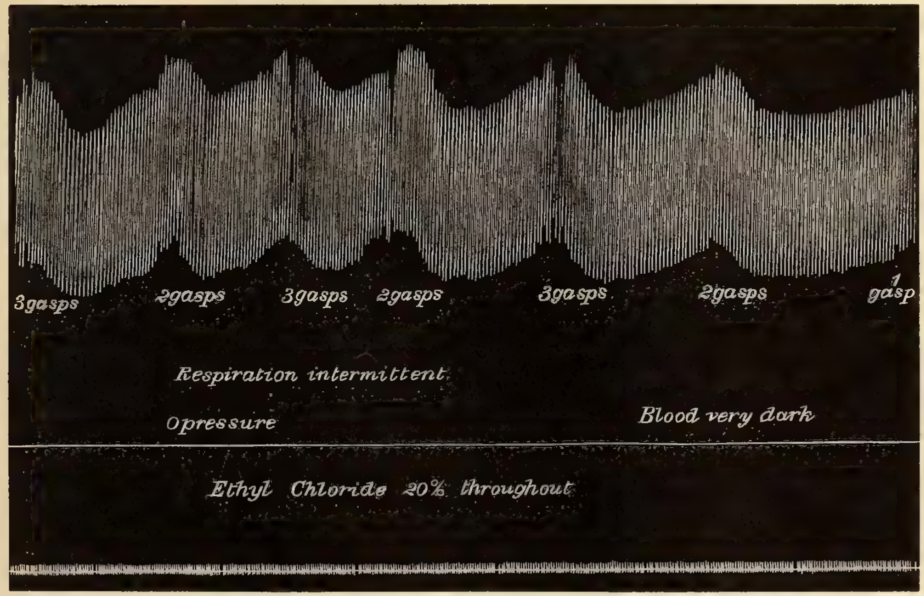

FIG. 11.—$\frac{3}{4}$ size of original.

In some experiments respiration failed independently of a fall in blood-pressure. In this case the respiration may remain in abeyance for considerable periods (6′ to 7′) without the blood-pressure being depressed, and subsequently continue in a normal manner.

The comparison of these results with those of chloroform (12) indicates that 10 per cent. of ethyl chloride vapour produces approximately the same effects upon the respiration as 2·5 per cent. of chloroform vapour in the air inspired. This quantitative relation is about the same as that which was found to obtain in the case of stimulation of the vagus mechanism by these two agents (p. 401).

In none of these experiments was it found that the heart was arrested before the respiration. Since, however, slowing of the heart usually began before the respiration showed signs of stoppage, it is possible that the heart

In the following table are collected a few observations upon the heart rate, respiration, blood-pressure, and corneal reflex from experiments with varying but known percentages of ethyl chloride in the air respired by natural respiration (except in one case):—

Dose.	Vagus inhibition of heart.	Respiration.	Fall of blood-pressure in mm. Hg.	Heart rate per minute.	Corneal reflex abolished.
Per cent.					
3·5	None in 15′	Unaffected in 15′	None in 15′	Unaffected in 15′	None in 15′
7	Slowing only	Unaffected in 13′	127 to 80 in 13′	90 to 69 in 13′	Gone in 2′ 4″
10	None	Slowing	Unaffected in 14′	Unaffected in 14′	,, 1′ 45″
10	Slowing only	Little irregular	198 to 50 in 14′ 30″	65 to 30 in 14′ 30″	,, 1′ 30″
10	Slowing down to arrest	Ceased as heart ceased	100 to 0 in 2′ 15″	60 to 0 in 2′ 15″	,, 1′ 30″
15	None	Unimpaired	Unaffected in 12′	Unaffected in 12′	,, 1′ 45″
15	Slowing down to arrest	Ceased twice for 2′ each	120 to 8	Slowing to stop, 74 to 0	,, 1′ 30″
18·5	,, ,,	Ceased in 8′ 10″	120 to 0 in 8′ 25″	65 to 0 in 8′ 25″	,, 1′ 15″
18·5	Slowing intermittently	Very slow	Unaffected in 14′	Intermittent rise and fall	,, 1′ 15″
20	,, ,,	,,	Falling after one inspiration, rising before the next	,, ,,	,, 1′ 30″
20	None	,,	78 to 70 in 13′	Unaffected in 13′	,, 1′ 45″
20	,,	Unimpaired	Unaffected in 12′	Unaffected in 12′	,, 1′ 10″
20	Slowing only	,,	124 to 90 in 7′ 30″	90 to 40 in 7′ 30″	,, 1′ 0″
20	Slowing	Ceased in 5′ 35″	135 to 56	68 to 24	,, 1′ 15″
30	Great slowing	Failed in 1′ 15″	120 to 24 in 1′ 15″	80 to 10 in 1′ 15″	,, 0′ 45″
30	Slowing intermittently	Very intermittent	116 to 18 in 8′ 40″	84 to 14 in 8′ 40″	,, 1′ 15″
30	Slowing down to arrest	Ceased at 0 blood-pressure	100 to 10 in 3′ 20″	86 to 0 in 3′ 20″	,, 0′ 35″
30	None	Unimpaired	102 to 38 in 7′	Unaffected	,, 1′ 10″
30	Great slowing	Failed in 2′ 45″	112 to 50 in 2′ 45″	90 to 10 in 2′ 45″	,, 0′ 50″
30	Arrested	Failed in 3′ 14″	124 to 0	80 to 0	,, 0′ 55″
30	Great slowing	Artificial respiration	104 to 30 in 4′ 30″	88 to 16 in 4′ 30″	,, 0′ 40″

may, in some instances in which there is exalted vagus irritability, stop before the respiration.

Conclusions.—In ethyl chloride narcosis the integrity of the respiratory mechanism is dependent upon the maintenance of blood-pressure.

Paralysis of respiration may be preceded by shallow respiratory movements, but it is usually preceded by deep and delayed breaths, and in some cases the prolonged pause between the expiration and the following inspiration prevents the fall of blood-pressure.

In all of the experiments performed the respiration failed before the heart.

CONCLUSIONS.

Conclusions with Regard to the Effects of Ethyl Chloride on Dogs.

(1) Quantities above 9 per cent. of ethyl chloride in the air respired exert a paralytic effect upon heart muscle similar to that produced by chloroform in $\frac{1}{19}$th the concentration.

(2) The central vaso-motor system is at first stimulated, and the peripheral mechanism of the arterioles is paralysed. The local paralytic effect is more significant than the central stimulation, so that the sum of these opposing factors is relaxation. This result is similar to that produced by chloroform, but the effect is never so profound, and requires for its production much higher concentration of ethyl chloride vapour.

(3) Its effect upon the vagus system is one of stimulation, and with high concentration the heart is readily arrested by vagus inhibition. As, however, the spontaneous excitability of the heart muscle is not seriously impaired, the heart escapes from vagus inhibition, and in no case has death occurred from this cause. To produce the same inhibitory effect with chloroform requires approximately a quarter the concentration in the respired air. Since it requires $\frac{1}{19}$th the concentration of chloroform to produce the same paralytic effect upon heart muscle, inhibition from ethyl chloride must ensue a variable period before the spontaneous excitability of the heart muscle is seriously impaired.

It is on this difference between the action of the two anæsthetics that the relative safety of ethyl chloride rests. The vagus system is not so readily depressed by prolonged administration as is the case with chloroform.

(4) Like chloroform the respiration under ethyl chloride narcosis is dependent upon the maintenance of the blood-pressure. The cause of the fall of blood-pressure from ethyl chloride is mainly vagus inhibition, whereas that from chloroform is cardiac paralysis completed by inhibition. Respiratory failure occurring apart from fall of blood-pressure, as, for

instance, in experiments with vagi cut, occurs within 15′ when the concentration of ethyl chloride exceeds 20 per cent.

In administrations not exceeding 15′ duration (these statements do not hold for periods exceeding this) in addition to the above-mentioned effect on the blood-pressure brought on through the vagus mechanism, the pressure falls from paralysis of the cardiac muscle when the concentration exceeds 20 per cent. The rate of fall of blood-pressure always varies with the rate of the respiration.

(5) 5 to 7 per cent. of ethyl chloride vapour in the air respired appears to be the limit of safety from syncope in dogs for prolonged and continuous administration. If this concentration be exceeded, it is inadvisable to continue the administration beyond abolition of the corneal reflex, otherwise syncope is very probable.

These conclusions with regard to the effects of ethyl chloride upon dogs likely apply to man. In man I have observed syncope occur in two instances and recovery to take place with a sudden return of pulse of fair volume and sudden replacement of the colour of death by that of life. From analogy with experimental results I regard such syncope as due to vagus inhibition. These conclusions, however, cannot be expected to hold good in clinical instances of failing heart and other morbid states that may embarrass recovery. A sound heart is able to free itself from vagus arrest, but the degree of depression caused by ethyl chloride may be sufficient during the period of syncope to embarrass an unsound heart beyond recovery.

To employ a gasometer with the ethyl chloride vapour mixed with air in the proportions required by the anæsthetist would be the most rational method of administration. The necessity of carrying a large bag for this purpose would, however, rob this anæsthetic to some extent of one of its advantages, viz., portability. The administration is rendered less safe by the employment of any apparatus for its administration in which the anæsthetic is sprayed or poured into the instrument between the face piece and the bag, so that during inspiration excessively high degrees of concentration of ethyl chloride vapour may be given. The anæsthetic should at least be allowed to mix with the air by being introduced at the end of the bag.

I acknowledge with much gratitude my indebtedness to Professor Osborne for his generous hospitality and assistance and for the use of his laboratory during the course of this investigation.

[*Note by Dr. C. J. Martin.*—Since Dr. Embley's paper was communicated to the Society, an investigation into the action of ethyl chloride, by Webster, has appeared in the 'Bio-Chemical Journal,' June, 1906, vol. 1, p. 328.

In Webster's experiments, as in Cole's, the quantity of ethyl chloride administered was not determined. This observer concludes that—(1) after a preliminary increase, the respiration rapidly diminished in rate and extent; (2) after a preliminary slight rise, the blood-pressure fell; (3) no paralysis of vagus endings occurs (in contradiction to Cole); (4) the action upon the circulatory system is, almost entirely, directly upon the heart.

Some of the records illustrating Webster's paper show marked vagus inhibition occurring during the course of the administration of the anæsthetic, but the author does not remark upon this fact.]

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EXPLANATION OF FIGURES.

FIG. 1 ($\frac{3}{4}$ size of original).—Isolated heart. Recovery. Dog. Weight, 8 kilogrammes. Morphine, 0·3 gramme and ether for anæsthesia in preparation. Common trunk of right carotid and subclavian arteries ligatured. The left carotid used for the monometer, and the aorta tied just beyond the left subclavian. The left subclavian artery is thus left open to carry the circulation. Its ascending branches and the vertebral arteries are then ligatured. Vagi cut. Artificial respiration. Blood-pressure tracing. Blood-pressure at starting 92 mm. Hg, 10 per cent. ethyl chloride vapour in the inspired air at starting "on" continued for $8' 15''$ and stopped at "off." The blood-pressure fell to 58 mm. The blood-pressure rose to 104 mm. in $9' 35''$ after administration ceased.

FIG. 2 ($\frac{3}{4}$ size of original).—Isolated heart. Recovery. Dog. Weight, 8·5 kilogrammes. Morphia, 35 grammes and ether for preparation as for experiment in fig. 1. Blood-pressure 158 mm. Hg at starting. Fifteen per cent. ethyl chloride vapour in the air respired started at "on" and continued for $3' 12''$ and stopped at "off." Blood pressure fell to 63 mm. Hg. In $2' 12''$ the blood-pressure had returned to 139 mm.

Fig. 3 ($\frac{3}{4}$ size of original).—Isolated heart. Recovery. Dog. Weight, 7·7 kilogrammes. Morphine, 0·32 gramme. Experiment similar to that of fig. 1, but 20 per cent. ethyl chloride was used in the air respired. Two experiments were performed. Started at "on" and stopped at "off" after 61″. Blood-pressure at start was 160 mm. Hg and fell to 70 in 1′ 30″. It recovered to 160 mm. in 1′ 25″ after stopping the administration. In 33″ after the recovery the administration was again started at the second "on" and continued for 59″. During that time the blood-pressure fell from 160 mm., reaching 62 mm. in 1′ 35″. It rose to 162 after administration ceased in 1′ 50″.

Fig. 4 ($\frac{12}{13}$ size of original).—Isolated heart. Recovery. Dog. Weight, 7·7 kilogrammes. Morphine, 0·32 gramme. Preparation as for experiment in fig. 1. Thirty per cent. ethyl chloride vapour in the respired air started at "on" and continued 46″. Blood-pressure, 164 mm. before starting, fell to 34 mm. in 1′ 34″. In 2′ 49″ it had risen again to 134 mm. Hg.

Fig. 5.—Double artificial circulation. Graphic representation of rate of blood flow through a piece of isolated bowel and isolated lungs. Defibrinated blood was circulated; 30 per cent. ethyl chloride vapour in the air was administered by artificial respiration to the isolated lungs. The curve plotted shows the increase of blood flow through the bowel circuit when the ethyl chloride was started.

Fig. 6 ($\frac{1}{2}$ size of original).—Effects of ethyl chloride upon the central nervous system. Bowel volume (B.V.) and blood-pressure (B.P.) figure. Dog, 8·4 kilogrammes in weight. Morphine, 0·3 gramme. Curare, 0·017 gramme. Vagi cut. Artificial respiration. Artificial arterial circulation to the brain, of defibrinated blood of another dog, containing ethyl chloride equal to 30 per cent. of vapour and at 38° C. The pressure was at that of the dog's femoral artery previous to the experiment. It was delivered by way of the two carotid arteries for periods of 19″, 23″, and 13″ in three successive experiments. In the first experiment the blood-pressure rose 57 mm., whilst at the same time the plethysmograph lever fell 6·5 mm. in the 19″ occupied by this artificial delivery. In the second experiment the blood-pressure fell 88 mm. and the lever rose 15 mm. In the third experiment the blood-pressure fell 40 mm. and the lever rose 27 mm. The first result was a constrictor, and the second and third were dilator effects.

Fig. 7 ($\frac{6}{7}$ size of original).—Organ volume measurements. Simultaneous measurements of bowel volume changes (B.V.) and blood-pressure (B.P.). Dog. Weight, 9 kilogrammes. Morphia, 0·4 gramme. Artificial respiration. Air containing 30 per cent. of ethyl chloride vapour administered between "off" and "on" for a space of 2′ 45″. A preliminary small rise of blood-pressure and fall of lever of the oncometer occurred. This was followed by a fall of blood-pressure and a rise in the lever of the oncometer, so that at 1′ 15″ the blood-pressure had fallen 46 and the tambour lever had risen 5 mm. But as the blood-pressure continued to fall the rise in the lever was not maintained and it began to fall, so that when the administration ceased the blood-pressure had fallen 65 mm. and the lever was 22 mm. below the lever at starting. In 3′ 5″ after cessation of administration the tambour lever had risen 42 mm., but the blood-pressure had only recovered 39 mm. The bowel volume was greater than it was before starting, although the blood-pressure was less. The bowel volume and blood-pressure were at their former levels in 13′ 25″.

Fig. 8 (size of original).—Vagus inhibition. Dog. Weight, 4·2 kilogrammes. Morphine, 0·2 gramme. Blood-pressure tracing. Air containing 10 per cent. of ethyl chloride vapour administered by artificial respiration. Blood-pressure at starting 83 mm. After 1′ 45″ administration the blood-pressure fell to 10 mm. from vagus inhibition. Recovery.

Fig. 9 (size of original).—Vagus inhibition from central stimulation. Dog. Weight, 9·4 kilogrammes. Morphine, 0·4 gramme. Curare, 0·02 gramme. Artificial respiration. Artificial arterial brain circulation prepared as in experiment for fig. 6, but with the vagi intact. Blood-pressure figure. Artificially circulating blood contained ethyl chloride equal to 30 per cent. of the vapour. Blood-pressure at starting 126 mm. Artificial circulation started at "on" and continued for 49″, ceasing at "off." Blood-pressure fell to 10 mm. The heart ceased from vagus inhibition. Recovery by vagotomy.

Fig. 10 ($\frac{4}{5}$ size of original).—Interdependence of respiration and blood-pressure. Dog. Weight, 7·8 kilogrammes. Morphine, 0·3 gramme. Respiration (Resp.) and blood-pressure (B.P.) figure. Air containing 30 per cent. ethyl chloride vapour started at "on" and administered continuously. Blood-pressure at starting 103 mm. Hg. In 3′ 54″ the blood-pressure fell to 10 mm. The respiration quickened after the commencement of the administration and slowed with the onset of heart slowing and ceased in 3′ 14″ with blood-pressure 35·5 mm. Hg.

Fig. 11 ($\frac{3}{4}$ size of original).—Respiration and blood-pressure. Dog. Weight, 7 kilogrammes. Morphine, 0·25 gramme. Blood-pressure figure. Ethyl chloride of unknown but large percentage administered by spraying the liquid into the mouth until the respiration ceased. Blood-pressure at starting "on," 118 mm. Some slowing from vagus inhibition occurred after 35″ of administration and lasted 1′ 26″, at which period the respiration, after slowing, ceased. Respiration continued in abeyance for 6′ 56″. Blood-pressure was 99 mm. at cessation of respiration and 98 mm. on its return.

The Anæsthetic and Lethal Quantity of Chloroform in the Blood of Animals.

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(Communicated by Dr. A. D. Waller, F.R.S. Received July 11,—Read November 1, 1906.)

(From the Physiological Laboratory of the University of London.)

Observations made *in vitro* on the relations which exist when liquid chloroform and defibrinated blood are in contact have shown that with equal concentrations of chloroform the tension of this in blood, serum or solutions of hæmoglobin, is very much lower than in water or saline solutions; a definite quantity of chloroform is associated with some constituent or constituents of the blood. When known weights of chloroform and blood are mixed together at 37° C., a percentage of 1·5 or more produces a precipitate of hæmoglobin. Determinations of the quantity of chloroform held by defibrinated blood have shown that for a given weight of chloroform this amount cannot be recovered by any of the methods which have been employed. The deficit may range from 2 to 20 per cent. We are of opinion that no experiment made *in vitro* can be regarded as an indication of what obtains when chloroform vapour is inhaled. The normal physiological conditions are not reproduced even when the vapour is in contact with defibrinated blood, which is apparently not the mode in which the majority of the experiments with which we are acquainted have been conducted, and still less are physiological conditions preserved when liquid chloroform is shaken up with blood.

Our observations were commenced in October, 1905, and carried out in the Physiological Laboratory of the University of London. We have employed an entirely new method of chloroform determination applied to the blood of anæsthetised animals—large cats were used for the majority of the experiments since the phenomena of anæsthesia in these animals closely resemble those in man (MacWilliam)*—and shall only refer briefly to the methods and results of those observers who have carried out such determinations of the quantity of chloroform found in the blood, with anæsthetic and lethal doses of this drug, as can be fairly compared with the results we have obtained.

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* 'Brit. Med. Journ.,' 1902.

In 1860, Lallemand Perrin and Duroy* investigated the quantity of chloroform in the blood and tissues of dogs killed with the anæsthetic. The chloroform in the blood was cleared out of this by heating in a current of air drawn through the blood, and the vapour was conducted through a red-hot glowing porcelain tube. The hydrochloric acid so formed was estimated by titration. They found 100 grammes of blood contained 1 c.c. of chloroform vapour.

In 1883, Gréhant and Quinquaud† published seven observations in which they determined the quantity of chloroform in the blood of dogs at the moment of anæsthesia, which was induced by the inhalation of 10 per cent. of chloroform vapour in air supplied by an apparatus which they had designed for administering variable amounts of chloroform. In their method the blood is removed without coming in contact with the air, and introduced into an apparatus for distilling *in vacuo.* At a temperature of 40° C., the blood gases are evolved, and at 65° C. the chloroform is distilled off. They state that almost all the chloroform in the blood is evolved as vapour with the blood gases. The receiver with the vapour and gases is washed out four to five times with water and added to the distillate. This liquid is sealed up in a tube, from which all the oxygen had been displaced by carbon dioxide, with Fehling's solution and heated. The amount of reduction was estimated by ascertaining the quantity of chloroform and water, a known weight of chloroform being used, which produced a similar amount of reduction of the same quantity of Fehling's fluid.

The quantities of blood used for these determinations were about 90 to 96 c.c., and the amount of chloroform in 1800 to 2181 grammes of blood was found to be 1 gramme. They concluded that about 50 milligrammes of chloroform per 100 grammes of blood is the anæsthetic dose, and this is only slightly less than the amount present in animals killed with chloroform.

Pohl in 1890‡ used a slight modification of the method which Schmiedeberg,§ who had adversely criticised the method employed by Lallemand Perrin and Duroy, introduced in 1867. Working with defibrinated blood and liquid chloroform, he determined the chloride produced when chloroform vapour is passed over glowing lime at a red heat. The calcium chloride is washed out of the tube with nitric acid, the liquid is neutralised with litmus and titrated with silver nitrate. The method employed by Pohl for determining the quantity of chloroform in blood during narcosis is similar, except that magnesia was substituted for lime.

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* 'Du Rôle de l'Alcool et des Anesthésiques dans l'Organisme,' Paris, 1860.

† 'Comptes Rendus des Séances de l'Académie des Sciences,' 9, vol. 97, p. 753, 1883.

‡ 'Arch. f. exp. Path. u. Pharmak.,' vol. 28, 1890–91.

§ 'Arch. f. Heilkunde,' p. 273, 1867.

The chief results of Pohl's determinations are that pure water in contact with chloroform takes up 0·794 per cent. at 15° C., which closely approximates to the figure obtained by Chancel and Parmentier,* 0·987 at 0° C., but is higher than the figures obtained by Moore and Roaf,† who find that at 17° C., with 0·04957 per cent. of chloroform vapour in a space where the vapour exerts a pressure of 74·61 mm. of mercury, the percentage by weight of chloroform taken up by distilled water is 0·586; that the amount recoverable from the blood of anæsthetised animals is much less than this, namely, 0·029, 0·018, 0·05 per cent. of chloroform, and that solutions of hæmoglobin in water do not take up more chloroform than the same volume of water, though an alcohol and ether extract of blood takes up as much as 1·105 per cent.

During narcosis in dogs Pohl obtained the following figures for arterial blood:—

	Grammes of $CHCl_3$ per 100 grammes of blood.
5 mins. after the commencement of administration ...	0·009
8 " " " " ...	0·018
60 " " " " ...	0·026
Death from chloroform	0·035
Blood from right ventricle	0·058
" left ventricle	0·027

In another experiment in which both the cardiac and respiratory movements had ceased in a dog weighing 32 kilogrammes, in the blood of the right ventricle, 0·042 gramme per 100, and in the blood of the left ventricle, 0·058 gramme per 100 of chloroform were found.

The chief conclusions which Pohl drew from his experiments were that:—

(1) The anæsthetic dose of chloroform in the arterial blood of dogs was 0·029 or 0·018 or 0·05 gramme of chloroform per 100 grammes of blood;

(2) The average amount which was present in the blood of animals killed with chloroform was 0·035 gramme per cent.

(3) The lethal and anæsthetic dose of chloroform closely approximate one to the other.

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* 'Comptes Rendus,' vol. 100, p. 773, 1885.

† 'Roy. Soc Proc.,' vol. 73, May 5, 1904.

The two experiments he records, which show the amounts of chloroform in the brain and blood of dogs killed with chloroform, are not concordant:—

	Grammes of chloroform in blood.	Grammes of chloroform in brain.
I	0·015	0·0418
II	0·043	0·036

The methods and mode of conducting the experiments detailed in Papers I and IV (appendices to the Report of the Special Chloroform Commission of the British Association)* do not come into consideration here since the work was chiefly undertaken with the view of ascertaining how far weighed amounts of liquid chloroform could be recovered from the tissues of small animals (Dodgson) or from the blood of animals anæsthetised or killed by chloroform, the blood being examined quantitatively for chloroform at periods varying from 1 to 10 days after the blood had been removed from the body (J. H. Wells).

Since the recent work of Tissot† and Nicloux‡ is directly comparable with that which we have undertaken, their method and results will be given in somewhat greater detail. The method of the French observers J. Mansion and Tissot,§ and also that employed by Nicloux, depends on Dumas' reaction, the production of potassium chloride when chloroform is treated with alcoholic potash. As applied to the estimation of chloroform in urine or blood outside the body, or blood withdrawn from different parts of the circulation, the method consists in distilling off the chloroform from defibrinated blood or other liquid which has been rendered acid with alcoholic solution of tartaric acid. The distillate is boiled with alcoholic potash, cooled, neutralised with sulphuric acid, using phenolphthalein as an indicator, and titrated with silver nitrate, using potassium chromate as an indicator (Nicloux). The error of the method averages 2 per cent. (Nicloux), while Tissot and Mansion show that 96·1 per cent. of the total chloroform added can be obtained from blood treated as above. The method adopted of distilling off chloroform from tissues after acidification is the usual toxicological method. One of us (J. A. G.) has been in the habit of frequently using this method of investigation in toxicological practice, and in his experience every trace of chloroform cannot be readily extracted and accurately estimated when only a few milligrammes are present. Without wishing to impugn in the slightest degree the accuracy of the control experiments of Nicloux, we are of opinion that the exact calculated and theoretical values found when working with 4 and 5 milligrammes of chloroform in 100 grammes of blood are due to accidental coincidence, more especially when we remember that the analytical process used involves distillation and subsequent saponification with alcoholic potash. We would also urge that even if it be granted as possible to estimate exactly by this method amounts of chloroform mixed with blood *in vitro,* the same accuracy cannot be expected with chloroform which has been introduced into the blood by the physiological process of inhalation; at the same time we must admit that his figures do not greatly differ from those in our experiments.

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* 'Brit. Med. Journ.,' July 11, 1902.

† 'Comptes Rendus,' No. 5, 1906.

‡ Nicloux, 'Comptes Rendus,' No. 2, 1906; No. 3, 1906; No. 7, 1906.

§ J. Tissot, 'Comptes Rendus,' No. 4, 1906.

The results obtained by Nicloux show for dogs:—

(1) That the anæsthetic dose varies for different animals.

(2) The amount in the arterial blood when anæsthesia is induced is about 50 milligrammes per 100 grammes of blood (confirmatory of Gréhant's results). In a dog weighing 18 kilogrammes, 58·5 milligrammes were found 60 minutes after commencement of administration, in another weighing 9·3 kilogrammes it was 47 milligrammes 33 minutes after the commencement of administration.

(3) The lethal dose in venous blood may be 70, 69, or 73 milligrammes per 100 grammes of blood.

(4) During the pre-anæsthetic period the intake of chloroform is rapid even with a low percentage of chloroform in the inspired air. Thus, within three minutes 56 milligrammes were found with a high percentage of chloroform in the air, and 55·5 milligrammes at the end of 32 minutes with a small percentage of chloroform.

(5) The differences between amounts of chloroform in arterial blood with an anæsthetic and lethal dose are small.

Anæsthetic dose. Milligrammes per 100 grammes.	Lethal dose. Milligrammes per 100 grammes.
54	70
57	64
57	69

An examination of Nicloux's figures, however, shows that in some cases the lethal dose was less than the anæsthetic one for the same dogs, thus—

Anæsthetic dose. Milligrammes per 100 grammes.	Lethal dose. Milligrammes per 100 grammes.
47	42
48	41

This is possibly due to alterations in the rate and depth of respiration. The weights of the dogs show that there is no relation between weight and an anæsthetic dose of chloroform—

Weight in kilogrammes.	Anæsthesia. Milligrammes per 100 grammes.
23	54
8	50
18	58
9	57

After anæsthesia has been induced and the supply of chloroform is stopped, this drug rapidly leaves the blood. Five minutes after anæsthesia half the amount disappeared; three hours later 7 milligrammes were found in the blood, which became free from chloroform seven hours after narcosis.

Using centrifugalised oxalated blood removed from the inferior vena cava, Nicloux recovered 64·4 per cent. of the chloroform from the red corpuscles and 13·3 per cent. from the plasma. However, the volume of the corpuscles was high in both the experiments he has described, being in one case 14 c.c. of plasma to 26 c.c. of deposit, and in the other 15 c.c. of plasma to 25 c.c. of corpuscles. His figures of the distribution of chloroform in blood are much below those of Pohl, who has stated that 87 to 90 per cent. of the total chloroform is held by the red corpuscles, that is the chloroform-holding power of the corpuscles is 7 to 8 times that of the plasma. The latter figures, as will be shown subsequently, are more in accord with our own observations.

The results obtained by J. Tissot show that:—

(1) 34 to 40 milligrammes of chloroform in 100 grammes of blood is sufficient to produce anæsthesia (Tissot), or 32 to 43 milligrammes (Mansion and Tissot).

(2) When mortal syncope is produced in dogs with very slow inhalation of small percentages of $CHCl_3$, the following amounts of chloroform are found in arterial blood:—

Milligrammes per 100 grammes.	Milligrammes per 100 grammes.
96	60
88·1	67·2
84·2	105·2
59·8	77·1

Just prior to mortal syncope the amount of chloroform in blood falls thus:—

Milligrammes per 100 grammes.	Milligrammes per 100 grammes.
65	62·2
62·5	56·05
54·5	

Tissot's deduction from this diminution in the blood which is constant just before death is that the choroform of the blood passes into and accumulates in the tissues, especially in the brain.

(3) The amount of chloroform in blood which produces anæsthesia varies with the rate of the induction of anæsthesia.

Mins.	Milligrammes of $CHCl_3$ per 100 grammes of blood.
2—3	60—70
5—8	44
Much slower	34, 35

(4) The determinations of the amount of chloroform found in venous blood after death by Nicloux is, according to Tissot, useless for a determination of the lethal dose, for this is always less than what is found in arterial blood, and when dogs are killed by the slow inhalation of 5, 6, 7, or 8 per cent. of chloroform vapour the lethal figure for arterial blood varies between 48 and 67 milligrammes. A quantity as large as 160 milligrammes may be found in blood before respiration stops. Such a large accumulation is impossible with inhalation of 4 to 5 per cent. chloroform vapour, for with this amount of chloroform it is impossible to raise the amount in arterial blood above 40 to 45 milligrammes per 100 grammes.

(5) Anæsthesia can exist with a very low content in the blood, for instance, 29 milligrammes per 100.

(6) There is no relation whatever between the proportion of chloroform in the blood and the effects of the drug, for these are determined, not by the absolute amount of chloroform present in blood, but by the quantity which enters the central nervous system. The amount in this may equal, never exceeds, and generally is much below that in the blood.

(7) During recovery from anæsthesia it has been constantly proved that the amount in venous blood exceeds that in the arterial. A study of the amount of chloroform in arterial blood should be made during the induction of anæsthesia and of the amount in venous blood during the disappearance of this condition.

	Cessation of chloroform administration immediately after anæsthesia.	45 minutes later.	2 hours later.
Arterial blood ...	53·2	5·8	0
Venous blood ...	48·1	7·7	4·9

The effect of chloroform upon the blood, which precipitates hæmoglobin and certain proteids of the serum (Formánek),* or the hæmolytic effect described by Harley† in 1865 with 5 per cent. of chloroform in blood, we have never observed in any of our experiments, since both the anæsthetic and lethal doses lie far below the quantity required to produce those effects.

Gréhant originally recognised that a combustible gas, which he believed was carbon monoxide, could be extracted from blood, and, in 1898, Desgrez and Nicloux‡ stated that normal blood contains 1 c.c. per cent. of total gases other than the normal, which they considered was carbon monoxide. During anæsthesia extending over five hours, the amount of this gas was found to be 2·5 c.c. per 1000 grammes of blood. Their recognition of this gas depends on the fact that carbon monoxide oxidised by anhydrous iodic acid at 150° C. yields carbon dioxide and free iodine. The latter is recovered, dissolved in chloroform and the tint of the solution estimated against a standard scale. Three experiments are described and from these it is concluded that chloroform is actually decomposed within the organism. We have obtained no evidence that any gas of this nature appears during chloroform narcosis, and since at least 80 per cent. of unchanged chloroform can be extracted from shed blood, it appears improbable that the remaining amount of chloroform in 1000 grammes of blood could yield anything like the quantity of 2·5 c.c. of carbonic oxide gas. They further state that in intense chloroform narcosis the proportion of carbonic oxide may even reach 6·9 c.c. per litre of blood.

If the contention of Desgrez and Nicloux is correct that as much as 6·9 c.c. of carbon monoxide may appear in 1000 grammes of blood during intense chloroform narcosis, about 1/25 of the total hæmoglobin would be saturated with this gas. The following experiment was made to determine whether any of the hæmoglobin is in the state of CO-hæmoglobin :—

Cat. Weight 3 kilogrammes.

2.30 P.M.	Ether anæsthesia commenced.
3 „	4 c.c. of blood withdrawn from carotid artery and defibrinated.
3.6 „	Chloroform inhalation commenced, the vapour being given by air drawn over liquid chloroform in a Woulff's bottle.
4.20 „	Anæsthesia with chloroform finished, inhalation pushed until respiration ceased. Blood to the amount of 75 c.c. was withdrawn and defibrinated. This was intensely dark.

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* Formánek, 'Zeitschr. f. physiol. Chemie,' vol. 29, p. 416, 1900.

† 'Phys. Soc. London Proc.,' 1865.

‡ 'Comptes Rendus,' p. 274, 1898.

No difference whatever could be detected, either spectroscopically or by using Haldane's method of examining very dilute solutions of blood in long glass tubes, between the condition of the hæmoglobin before and after 1¼ hours' continuous chloroform narcosis.

However, positive information that CO-hæmoglobin is present can be at once obtained by the above methods when 0·5 c.c. pure CO was added and shaken up with 73 c.c. of the same blood which had failed to give any indication of CO-hæmoglobin. We are therefore unable to accept the view that the combustible gas stated to appear during chloroform narcosis is carbon monoxide, a product of the decomposition of chloroform within the organism. The recognition of CO-hæmoglobin is quite easy when 1/10 to 1/20 of the total colouring matter of the blood has taken up carbon monoxide.

I.—*Determination of the Amount of Chloroform in Blood.*

In the experiments described in this paper the amount of chloroform in the blood was calculated from the difference in the chlorine-content of the blood before and after administration of the anæsthetic. The validity of this mode of estimation depends, of course, on the constancy of the natural chlorine-content of the blood during the whole course of an experiment. We fully satisfied ourselves, however, by a series of preliminary observations, some of the results of which are given later, that under the conditions of all our experiments the percentage of natural chlorine did remain sufficiently constant.

Method.—The animal experimented on was anæsthetised by ether and the necessary operations for introducing cannulæ into the carotid artery and the trachea were performed. The tracheal cannula was fitted to a Chauveau's valve and a side-tube in connection with apparatus for recording the rate and character of the respirations. Chloroform was administered sometimes by means of a Woulff's bottle, sometimes from bags filled with mixtures of air and chloroform vapour of known composition by means of the Dubois apparatus. The samples of blood were taken from the carotid artery and collected in stoppered weighing tubes. As a rule the ether was cut off after the operation had been performed, and the animal allowed so far to recover that the conjunctival reflexes reappeared and the anæsthesia was light. A control sample was then taken in order to determine the percentage of natural chlorine. After this, chloroform was administered and samples of blood were taken during various stages of anæsthesia. No blood-pressure observations were taken, as we thought it desirable to interfere as little as possible with normal physiological conditions. During the experiments on any particular animal, which sometimes lasted two hours or longer, every effort was made, by keeping a close watch on the animal, to prevent any sudden fluctuations in the rate of administration—for instance, if the animal held its breath the chloroform was not pushed. In no experiment did the animal lose any blood beyond what was taken for the samples, except in certain experiments deliberately undertaken to test the effect of loss of blood.

Mode of Estimating the Chlorine.—After careful consideration of the various available methods for estimating chlorine, we selected, owing to the volatility of chloroform, the well-known method of Carius, commonly used for the determination of the amount of halogen in organic compounds. The stoppered weighing tube, containing, as a rule, from 5 to 6 grammes of blood, was placed along with 6 to 8 c.c. of fuming nitric acid (1·5), and an excess of solid silver nitrate in a bomb tube, which was then sealed. The bomb was then heated in the furnace to 150° C. for six hours. With the quantities of material used, this time and this temperature proved quite sufficient to completely oxidise the organic matter. The bomb was then opened and the silver chloride formed was weighed. It is generally considered that chlorine can be estimated with great exactness by the gravimetric method, using paper filters and, according to Fresenius, we can, with care, always obtain 99·9 to 100·1 for 100 parts of chlorine taken. By adopting Gooch's method of filtration and J. P. Cooke's suggestion of washing with water containing a little silver nitrate, by which the small minus error caused by the slight solubility of silver chloride in water is obviated, and a few other simple precautions, a much higher degree of accuracy can be attained.

As the quantities of silver chloride weighed in our experiments were very small—varying from 0·03 to 0·09 gramme—we thought it necessary to adopt all possible precautions to obviate the slight errors, which, when large quantities are weighed, are for ordinary practical purposes negligible.

The contents of the bomb tube were transferred to a beaker, diluted with water and heated to the boiling temperature for a few minutes. After cooling, the liquid was filtered through the Gooch, and the silver chloride washed into the crucible by means of hot water containing a little nitric acid and silver nitrate. The washing was completed with cold water. The crucible and its contents were then dried at 140° C. and weighed. The silver chloride was then dissolved on the filter by means of strong ammonia, and the asbestos washed with ammonia until free from silver. The crucible was then finally washed with hot water, dried as before, and weighed. The difference between the two weighings, (1) crucible + silver chloride + possible glass fragments and (2) the crucible + fragments, gave the absolute amount of chloride present.

It was found more convenient to obtain the weight of the silver chloride in this way, rather than directly by filtering through an already weighed crucible, as it was almost impossible to prevent traces of glass dust getting into the silver chloride during the heating and during the opening of the bomb. This plan was, therefore, adopted in many of the experiments, but in any case the result was always checked by dissolving out the silver chloride in the manner described. We believe this plan far preferable in our case to that usually followed of dissolving the silver chloride away from the glass and then reprecipitating with nitric acid.

Control Experiments.—In order to ascertain whether the percentage of chlorine in the blood of an animal remains sufficiently constant during prolonged anæsthesia accompanied by periodical bleeding, a number of experiments were made with cats, which conclusively showed that under the conditions of our experiments the percentage does remain constant. In illustration we quote the following experiments:—

Experiment 1.—A cat, weighing 3·2 kilogrammes, was anæsthetised by ether and samples of the blood taken at intervals of ten minutes, the character of the respirations being recorded on a drum. Every effort was made to keep the anæsthetic condition of the animal as constant as possible during the experiment. It was found that the colour of the blood afforded a good index of the degree of the anæsthesia. Six samples of blood were taken, after which chloroform was administered until the animal was asphyxiated, when a seventh sample was taken. The results of the analysis are given in the following table—Table I. The colour of the blood after respiration ceased was a deep brownish-red, such as we have always found to be the case in chloroform narcosis at this stage, though the oxygen-carrying property of hæmoglobin is known not to be impaired by chloroform.

Experiment 2*a*.—A pregnant cat, weighing 3·3 kilogrammes, was anæsthetised as before, and samples of blood taken. The experiment was somewhat similar to No. 1, except that the intervals between the abstraction of the various samples were longer. Five samples were taken, after which chloroform was administered until the animal was very deeply under the influence of the drug, but was still breathing satisfactorily.

The results are given in Table IIa.

Experiment 2*b*.—A cat, weighing 2·5 kilogrammes, was etherised. Four samples of blood were collected for analysis, but between the first and second samples 10 c.c. of blood were withdrawn, and between the third and fourth 5 c.c. After the fourth sample was taken the animal was chloroformed, using a Woulff's bottle, until respiration ceased.

The results are given in Table IIb.

Table I.—April 26, 1906. Cat under Ether. Weight, 3·2 kilogrammes.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Respirations per minute.	Remarks.
P.M. 2.15	Ether began								
2.55	Ether	5·1568	0·0692	0·33079	0·331396	−0·00061	0·001123	23	Reflexes just visible. Blood medium colour
3.5	"	5·2274	0·0698	0·32916		−0·00224		23	Ditto
3.15	"	4·3200	0·0583	0·33267		+0·00127		23	Colour slightly brighter
3.25	"	4·6081	0·0619	0·33114		−0·00026		—	Colour slightly brighter. Cheyne Stokes' respirations
3.35	"	5·2557	0·0706	0·33113		−0·00027		24	Much same
3.45	"	4·4572	0·0603	0·33349		+0·00209		24	About same
3.46	Chloroform put on, using Woulff's bottle, with entrance tube under $CHCl_3$, and continued until the animal was asphyxiated. A sample of blood was now taken, which was deep red in colour. It did not smell noticeably of chloroform							17	Reflexes gone Cessation of respiration
		6·9746	0·1035	0·36581		0·0344 as chlorine 0·0386 as chloroform			
			= 7·7 c.c. as vapour of chloroform at 0° C. and 760 mm.						

Table IIa.—Pregnant Cat. Weight, 3·3 kilogrammes. Ether.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
A.M. 11.0	Ether started							
11.35	Ether	3·6765	0·0518	0·34732	0·3478	−0·00048	0·00165	1st sample taken
11.50	,,	4·5590	0·0641	0·34659		−0·00021		2nd sample, colour same
P.M. 12.13	,,	5·2646	0·0740	0·3465		−0·0013		3rd sample, colour similar
12.28	,,	4·6735	0·0668	0·35244		+0·0046		4th sample, colour same
12.43	,,	6·0244	0·0846	0·34617		−0·0016		5th sample, colour same
12.44	Chloroform was now administered by means of a Woulff's bottle, and at 12.56, when the animal was fairly deeply under, a sample of blood was drawn. It was rather dark in colour.							
		5·5070	0·0821	0·3675		0·0197 as chlorine 0·0222 as chloroform		
		= 4·15 c.c. as vapour of CHCl$_3$ at 0° C. and 760 mm.						

Table IIb.—Cat. Weight, 2·5 kilogrammes.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
A.M. 11.30	Ether started							
P.M. 12.7	Ether	5·2180	0·0729	0·34439	0·34135	+0·00304	0·00166	1st sample, 10 c.c. extra drawn
12.8	,,							
12.35	,,	4·3222	0·0597	0·34049		−0·00086		2nd sample
12.50	,,	4·2139	0·0584	0·34163		+0·00028		3rd sample, 5 c.c. drawn for another purpose
12.57	,,							
1.14	,,	4·9247	0·0677	0·33888		−0·00247		4th sample
1.19	Chloroform was now slowly admininistered, more rapidly towards the end, by means of a Woulff's bottle, until respiration ceased. Sample was very dark in colour.							
		6·2537	0·0947	0·37329		0·03194 as chlorine 0·03586 as CHCl$_3$		
		= 6·73 c.c. as vapour of CHCl$_3$ at 0° C. and 760 mm.						

Experiment 3.—We quote this experiment as an illustration of the greatest difference in the chlorine content of the blood ever noticed. In this particular experiment a large quantity of blood, about 23 c.c., was withdrawn during the interval between the times of taking the samples analysed. The cat weighed 3·8 kilogrammes. The results are given in Table III.

Table III.—April 13, 1906. She Cat. Weight, 3·8 kilogrammes.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	$CHCl_3$ per cent.	Mean value of chlorine per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
P.M. 12.15	Ether on							
12.48	”	4·2378	0·0572	0·33273	0·33798	−0·00525	0·00525	
12.50	”	—	—	—		—		18 c.c. blood taken from animal, making altogether about 23 c.c.
1.4	”	3·9088	0·0543	0·34323		+0·00525		Animal nearly asphyxiated. It could not be brought round

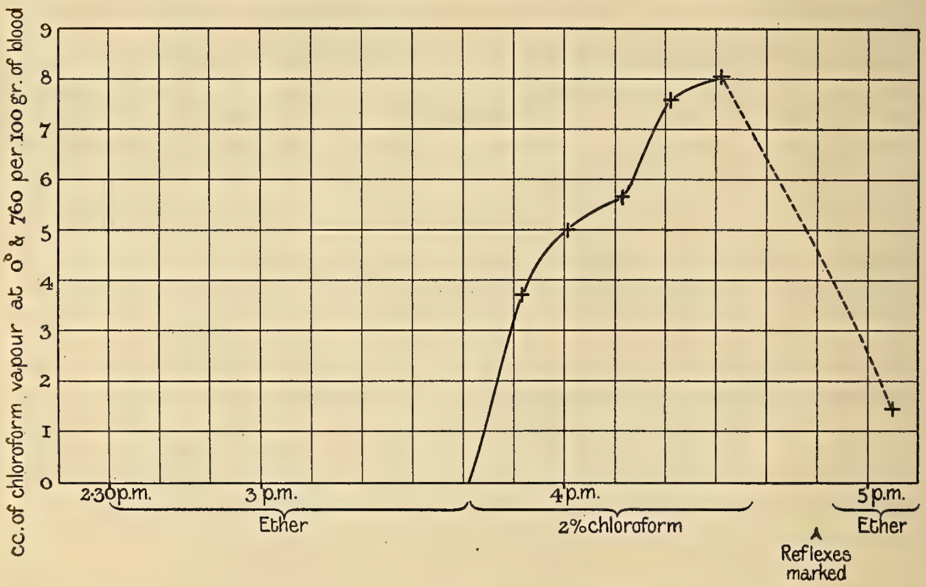
Experiments to Determine the Amount of Chloroform in the Arterial Blood of an Animal at Different Stages of Chloroform Narcosis.

Experiment 4.—This was one of the earliest experiments performed. The blood was taken from the left carotid. The chloroform was administered by means of a Woulff's bottle, and after the animal was fairly deeply anæsthetised it was kept as far as possible under constant conditions. The results are given in Table IV.

Table IV.—Cat. Left carotid tapped.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Difference from control—			Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.	
P.M. 12.30	Ether started							
1.0	Ether	5·0843	0·0677	0·3282	—	—	—	Sample taken when reflexes just gone
1.0½	Chloroform on							
1.9	”	3·8387	0·0534	0·3429	0·0147	0·0165	2·56	Reflexes going
1.14	”	4·5512	0·0645	0·3493	0·0213	0·024	4·5	Animal maintained in the same state of anæsthesia throughout
1.19	”	5·6276	0·0796	0·3487	0·0206	0·023	4·3	

Experiment 5.—In this experiment a male cat, weighing 4 kilogrammes, was used, and the blood was taken from the right carotid. The chloroform was administered from bags filled by means of the Dubois apparatus, with approximately a 2-per-cent. mixture of chloroform vapour and air. The animal was gradually anæsthetised until respiration ceased, when the chloroform was cut off and the cat allowed partially to recover. It was then kept under light ether anæsthesia until 5.5 P.M., 29 minutes after the chloroform was stopped, when a final sample of blood was drawn. The samples of blood while the animal was under chloroform were taken every 10 minutes, and the respirations were recorded. The darkness of the colour of the blood increased with the percentage of anæsthetic in the blood, and appeared to afford a good criterion of the progress of the anæsthesia. The results of this experiment are given in Table V and Curve A. After the chloroform was cut off the anæsthetic was rapidly eliminated from the blood, and 14 minutes later the reflexes were well marked. At the time of taking the final sample the greater portion of the chloroform had disappeared.



A.

Constructed from Experiment 5. Samples of arterial blood (+).

Experiment 6.—For this experiment a female cat, weighing 4·3 kilogrammes, was taken. The right carotid was tapped, and the chloroform administered by means of a Woulff's bottle. The control sample was taken when the reflexes were just visible. The chloroform was then administered, and samples

Table V.—Cat. Weight, 4 kilogrammes. Chloroform administered by Dubois Apparatus. Right carotid tapped.

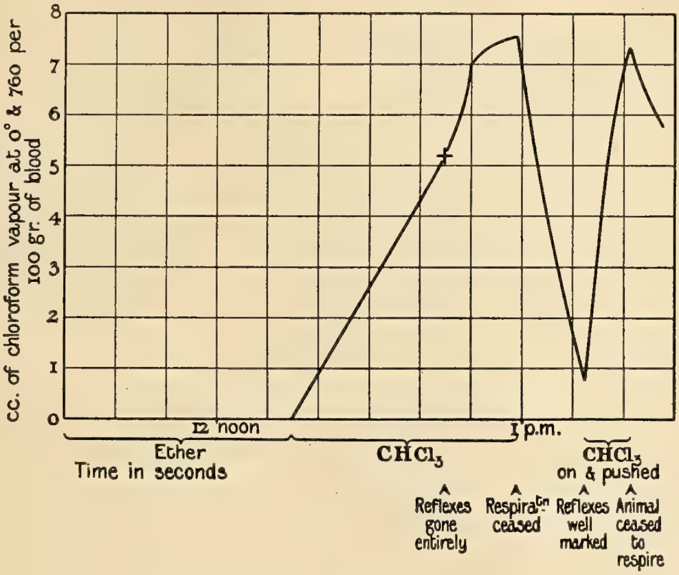
Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Differences from control—			Colour.	Respirations per minute.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
P.M. 2. 30	Ether put on.									
3. 41	Ether off	4 ·9281	0 ·0667	0 ·3336	—	—	—	Fairly bright ...	30	Convulsive movements
3. 41	Chloroform on, 2 per cent.									
3. 51	„ „	4 ·4422	0 ·0633	0 ·3513	0 ·0176	0 ·0198	3 ·71	Slightly darker...	19	
4. 1	„ „	4 ·1523	0 ·0602	0 ·3574	0 ·0237	0 ·0266	5 ·0	„ ...	18	
4. 11	„ „	4 ·4326	0 ·0648	0 ·3604	0 ·0267	0 ·03	5 ·63	Slightly deeper...		
4 ·21	„ „	4 ·3817	0 ·0657	0 ·3696	0 ·036	0 ·0404	7 ·58	Much same	36	Deep respirations
4. 31	„ „	5 ·8127	0 ·0877	0 ·3719	0 ·0384	0 ·0431	8 ·09	Rather lighter ...	30	Sample difficult to get, squeezing resorted to
4. 36	Chloroform off.									
4. 50	....	—	—	—	—	—	—	—	—	Reflexes marked
4. 53	Ether on.									
5. 5	Ether on	4 ·6707	0 ·0645	0 ·3404	0 ·0068	0 ·0076	1 ·4	Very bright	20	

Table VI.—Cat. Weight, 4·3 kilogrammes. Right carotid tapped. Chloroform administered from Woulff's bottle.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Difference from control—			Colour.	Remarks.
					As chlorine.	As chloroform.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
A.M. 11.30	Ether started								
P.M. 12.14	Under ether	5 ·6099	0 ·0754	0 ·3313	—	—	—	Medium......	Reflexes just reappearing
12.14	Chloroform on instead of ether, light anæsthesia								
12.45	" "	3 ·8512	0 ·0556	0 ·3559	0 ·0246	0 ·0276	5 ·17	Medium......	Eye and tail reflexes just disappeared entirely
12.50	" "	3 ·5211	0 ·0521	0 ·3647	0 ·0334	0 ·0375	7 ·04	Rather dark	Breathing satisfactory
12.59	" "	4 ·0755	0 ·0607	0 ·3671	0 ·0358	0 ·0402	7 ·55	Very dark ...	Respiration just ceased
12.59	Chloroform stopped..........	—	—	—	—	—	—	—	Artificial respiration resorted to
1.2	....	—	—	—	—	—	—	—	Breathing began naturally
1.12	....	4 ·2136	0 ·0573	0 ·3352	0 ·0039	0 ·0044	0 ·82	Very bright	Reflexes just reappeared
1.13	Chloroform on and pushed								
1.21	" "	4 ·8972	0 ·0727	0 ·3659	0 ·0346	0 ·0389	7 ·30	Very dark ...	Animal on point of asphyxiation

The animal was brought round again by use of the bellows, and a sample of blood, taken when reflexes just reappeared, was bright.

taken when the conjunctival and tail reflexes had entirely disappeared, five minutes later, and when respiration just ceased. The chloroform was stopped at this point, and the animal allowed to recover. In this case artificial respiration had to be resorted to before the cat began to breathe naturally. A sample of blood was collected when the reflexes had just entirely reappeared. After this stage chloroform was again administered until the animal was on the point of asphyxiation, when the final sample of blood was taken. It will be noticed that in this experiment also the colour of the blood kept pace closely with the amount of anæsthetic in the blood. After the final sample had been taken the animal was revived again by the use of the bellows, and the blood appeared bright in colour. The amounts of chloroform per cent. of blood were practically identical at the two points where respiration ceased. The results of the experiment are recorded in Table VI and Curve B.



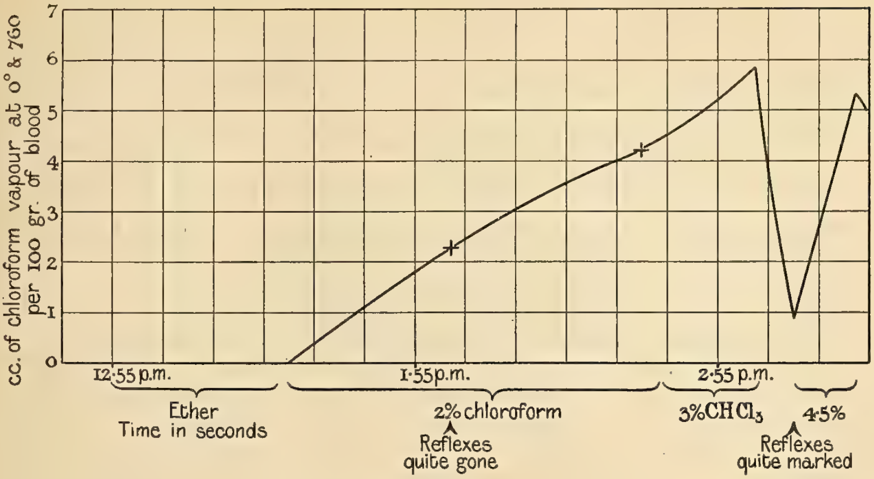
B.

Constructed from Experiment 6.

Experiment 7.—In this case a male cat, weighing 3·5 kilogrammes, was taken and the right carotid tapped. The chloroform was administered from bags filled with mixtures of chloroform vapour and air by means of the Dubois apparatus. At first 2-per-cent. chloroform mixture was used, and samples of blood were drawn after the reflexes had entirely vanished and when the animal was very deeply under. Three-per-cent. chloroform mixture was then substituted for 2-per-cent., and a sample taken when the animal

Table VII.—Cat (male). Weight, 3·5 kilogrammes. Blood taken from right carotid. Chloroform administered by Dubois apparatus.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Differences from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
P.M. 12.55	Ether started								
1.27	Under slight ether	4·0709	0·0547	0·3313	—	—	—	Fairly bright...	Reflexes just reappeared
1.28	Chloroform on, 2 per cent. approximately								
1.33	Chloroform off	—	—	—	—	—	—	—	Breathing unsatisfactory
1.55	2 per cent. chloroform on								
2.2	Continued	3·9485	0·0548	0·3421	0·0108	0·0121	2·27	Slightly darker	Respiration, 20 per min.
2.21	2 per cent. continued	—	—	—	—	—	—	—	„ 18 „
2.30	„ „	—	—	—	—	—	—	—	„ 23 „
2.39	„ „	—	—	—	—	—	—	—	„ 21–22 per min.
2.40	„ „	3·8731	0·0551	0·3509	0·0196	0·0223	4·18	Deeper	
2.44	3 per cent. chloroform on								
2.48	Continued	—	—	—	—	—	—	—	Respiration shallow and very irregular, about 40 per min., evidently the Cheyne-Stokes type of breathing. This continued, and was very clearly marked
2.59	„	4·2182	0·0614	0·3588	0·0275	0·0309	5·79	Very deep	
3.2	Chloroform off	—	—	—	—	—	—	—	Animal began to die; after discontinuance of $CHCl_3$ it recovered naturally
3.10	....	4·0592	0·0553	0·3358	0·0045	0·005	0·94	Fairly bright...	Reflexes well marked
3.11	4·5 per cent. $CHCl_3$ on								
3.15	„ „	—	—	—	—	—	—	—	Respirations, 20 per min., animal difficult to get under
3.19	„ „	—	—	—	—	—	—	—	Reflexes not quite gone, and clonic twitches of hind limbs
3.22	„ „	4·4367	0·0641	0·3561	0·0248	0·0279	5·23	Very dark indeed	Movements vanished and respiration almost ceased



C.

Constructed from Experiment 7.

showed signs of asphyxiation. During the administration of the 3-per-cent. mixture the respiration was shallow and irregular, and of the Cheyne-Stokes type. The animal was then allowed partially to revive, and a sample of blood was collected when the reflexes were well marked. Chloroform was now administered again, the mixture of chloroform vapour and air used being of approximately 4·5 per cent. strength. The reflexes took rather a long time to entirely disappear, and the time of disappearance was rendered more difficult to ascertain by the complication of clonic twitching of the hind limbs. These movements vanished before respiration ceased, when a sample of blood was collected.

The results of the experiment are given in Table VII and Curve C. It will be noticed that the amounts of chloroform in the blood at the two stages of the experiment at which respiration ceased are almost identical.

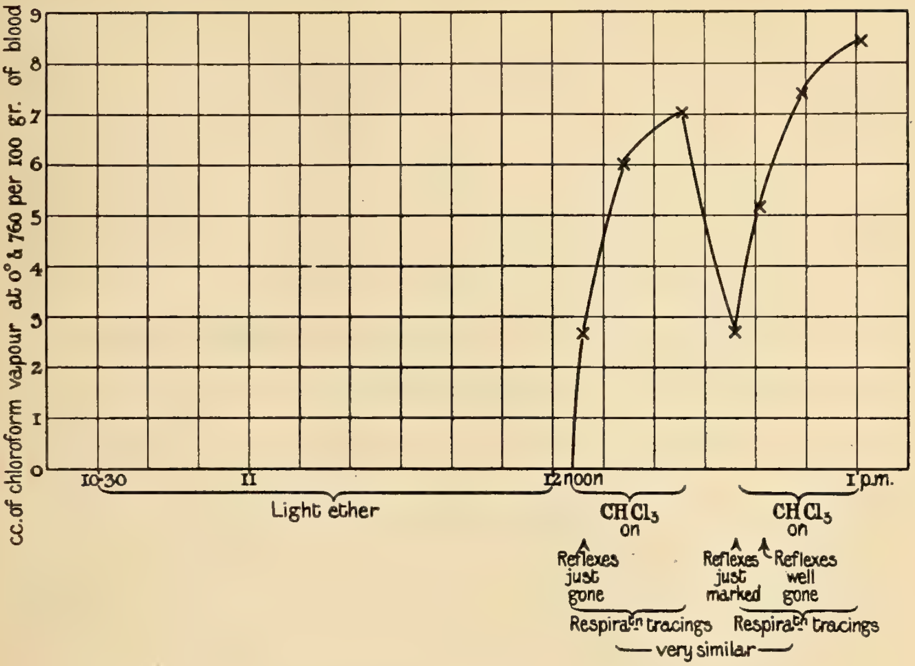
Experiment 8.—In this experiment a female cat, weighing 2·7 kilogrammes, was taken and the chloroform was administered from bags filled with air, containing approximately 2 per cent. of chloroform vapour, by means of the Dubois apparatus. The object of this experiment was to obtain two curves showing in the one case the proportions of chloroform in the blood at various stages up to the asphyxiation point immediately after anæsthetisation by ether, and in the other case the amounts at the various stages in a second chloroform experiment, after the animal had recovered from the first.

After the control sample had been taken under ether, the cat was allowed so far to recover that the reflexes were well marked, and then the chloroform was administered. Samples of blood were collected when the reflexes had

Table VIII.—Cat (female). Weight, 2·7 kilogrammes.

(In this experiment the chloroform was administered by Dubois apparatus, and was approximately 2 per cent. strength.)

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Per-centage of chlorine.	Difference from ether control—			Colour.	Respirations per minute.	Remarks.
					As chlorine.	As chloro-form.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M. 10.30	Ether on, light anæsthesia	—	—	—	—	—	—	—	28	
11.58	....	4·1885	0·0578	0·3402	—	—	—	Fairly bright	—	Reflexes just visible (eye and tail)
11.59	Chloroform on ...	—	—	—	—	—	—	—	22	
12.0	Chloroform off.									
P.M. 12.3	" ...	—	—	—	—	—	—	—	—	Reflexes marked
12.4	Chloroform on.									
12.6	" ...	5·4454	0·0779	0·3526	0·0125	0·014	2·63	Very bright ...	24	Reflexes just gone.
12.14	" ...	4·1395	0·0619	0·3686	0·0284	0·0319	6·0	Darker	22	Animal very deeply under, but breathing satisfactorily
12.26	" ...	4·6527	0·0705	0·37352	0·0333	0·0374	7·03	Much darker	—	
12.27	Chloroform off.									
12.33	" ...	—	—	—	—	—	—	—	34 (frequent, deep)	Eye reflexes just beginning
12.35	" ...	—	—	—	—	—	—	—	—	Slight tail reflex appears
12.36	" ...	5·1162	0·0732	0·3527	0·0125	0·01406	2·64	Bright.		
12.37½	Chloroform on.									
12.39	" ...	—	—	—	—	—	—	—	—	Reflexes began to go
12.41	" ...	4·7880	0·0708	0·3645	0·0243	0·0273	5·13	Darker	39	Reflexes completely gone
12.49	" ...	6·3007	0·096	0·3756	0·0354	0·0398	7·46	Much darker...	40 (shallow)	Animal very deeply under, but breathing satisfactorily
1.1	" ...	5·2791	0·0814	0·3801	0·0399	0·0448	8·42	About same ...	20	



D.

Constructed from Experiment 8. Samples of arterial blood (+).

just disappeared, when the animal was moderately deeply under, and when respiration was stopping. The animal was then allowed partially to revive, and a sample was taken when the reflexes were well marked. Chloroform was now administered again, and samples were collected when the reflexes had *just* disappeared, when the animal was deeply under, and when respiration began to cease, the various points at which the blood was taken being selected so as to correspond as far as we could judge with those in the first part of the experiment.

The results of the experiment are given in Table VIII and Curve D. It will be noticed that the two portions of the curve correspond in form, but the second part is slightly shifted in an upward direction.

Experiment 9.—The object of this experiment was to test the effect on the amounts of chloroform necessary to produce various anæsthetic phenomena of taking away a large proportion of the animal's blood, which is undoubtedly the vehicle through which chloroform is enabled to produce its effects on the animal. For this purpose a female cat, weighing 3·3 kilogrammes, was selected, and the chloroform was administered by means of a Woulff's bottle, in which the height of the air inlet tube above the chloroform could be varied readily. After the control sample of blood had been withdrawn, the animal

Table IX.—Cat. Weight, 3·3 kilogrammes.

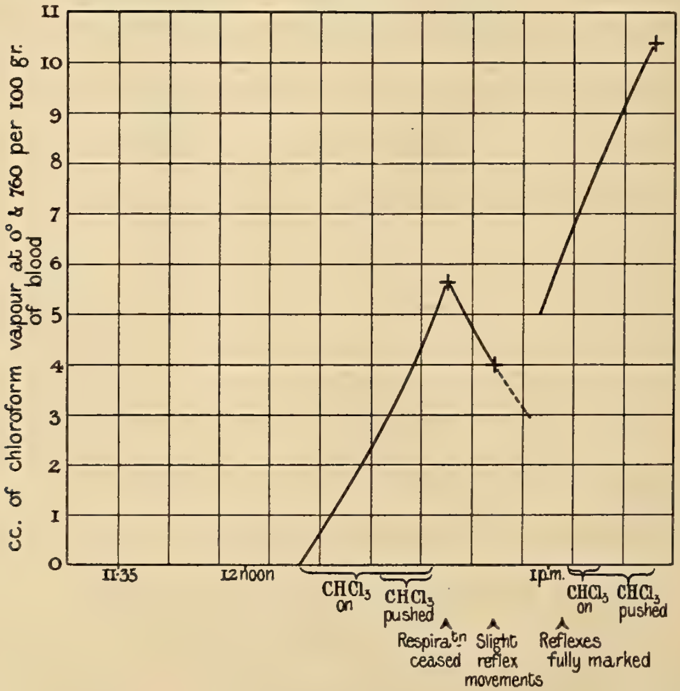
Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Per-centage of chlorine.	Differences from control—			Colour.	Respira-tions per minute.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M. 11.35	Ether put on.									
P.M. 12.5	Ether off.									
12.7	....	—	—	—	—	—	—	—	28	
12.10	....	—	—	—	—	—	—	—	—	Good reflexes
12.10½	....	4·1749	0·0577	0·3407	—	—	—	Somewhat dark		
12.11	Chloroform on	—	—	—	—	—	—	—	28	
12.14	”	—	—	—	—	—	—	—	36	
12.22	”	—	—	—	—	—	—	—	—	Alternating rhythmical movements of hind limbs
12.24	”	—	—	—	—	—	—	—	—	” ”
12.25	”	—	—	—	—	—	—	—	—	” ”
12.27	Chloroform pushed.									
12.30	” ”	—	—	—	—	—	—	—	44	
12.33	” ”	—	—	—	—	—	—	—	52	
12.35	” ”	—	—	—	—	—	—	—	80	” ”
12.37	” ”	—	—	—	—	—	—	—	16	
12.40	Chloroform off	4·7989	0·0715	0·3673	0·0266	0·0299	5·61	Very dark	—	Animal revived naturally
12.42	....	—	—	—	—	—	—	—	28	Respiration deep
12.46	....	—	—	—	—	—	—	—	28	”
12.47	....	—	—	—	—	—	—	—	—	Reflexes began very slightly
12.49	....	5·2056	0·076	0·3599	0·0192	0·0216	4·05	Bright red		
12.50	....	—	—	—	—	—	—	—	—	32 c.c. blood withdrawn, making total since control of about 47 c.c.

12.52	....	—	—	—	—	—	—	—	24	
1.3	....	—	—	—	—	—	—	—	—	Reflexes fully back
1.4	Chloroform on	—	—	—	—	—	—	—	24	
1.6	„	—	—	—	—	—	—	—	24	Reflexes vanished
1.8	„	—	—	—	—	—	—	—	28	
1.10	Chloroform pushed	—	—	—	—	—	—	—	32	
1.11	„ „	—	—	—	—	—	—	—	40	
1.13	„ „	—	—	—	—	—	—	—	—	Rhythmical alternate leg movements began
1.14	„ „	—	—	—	—	—	—	—	56	„ „
1.16	„ „	—	—	—	—	—	—	—	80	„ „
1.17	„ „	—	—	—	—	—	—	—	48	
1.18	„ „	—	—	—	—	—	—	—	64	Faint and shallow respiration
1.19	„ „	—	—	—	—	—	—	—	64	„ „
1.19½	„ „	—	—	—	—	—	—	—	—	Respiration stopped
1.21	„ „	5 ·6528	0 ·0894	0 ·3899	0 ·0492	0 ·0552	10 ·37	Very dark		

was allowed to recover until the ether anæsthesia was very light. The chloroform bottle was now connected to the Chauveau valve, and the anæsthetic administered fairly gently for 14 minutes, after which it was pushed by lowering the inlet air tube to the surface of the chloroform, and a sample of blood was collected when respiration ceased; the blood at this stage was very dark in colour. During the period in which the anæsthesia was pushed curious alternating rhythmical movements of the hind limbs were noticed. The cat was allowed to recover naturally, and a sample of blood collected when the reflexes were slightly marked. The colour of the sample was bright red. Thirty-two cubic centimetres of blood were now withdrawn from the animal, making in all since the control sample about 47 to 50 c.c. This quantity was probably a little over 1/3 of the total blood in the animal.

The chloroform was now administered again, and, after the first few minutes, was pushed until respiration stopped, when a sample, very dark in colour, was collected. During the later stages of this anæsthesia, the above-mentioned rhythmical movements of the limbs were again noticed, and they ceased a little before respiration stopped.

The results of this experiment are recorded in Table IX and Curve E. It will be noticed that the amount of chloroform in the blood at the asphyxia-



E.

Constructed from Experiment 9. Samples of arterial blood (+).

tion stage was very much higher in the second half of the experiment than in the first. In connection with this experiment we would draw attention to the results of the administration of chloroform recorded at the end of Tables I and II.

In the two following experiments we used dogs instead of cats. The phenomena observed were similar to those in the case of cats, but the percentages of chloroform found at the various stages were somewhat higher.

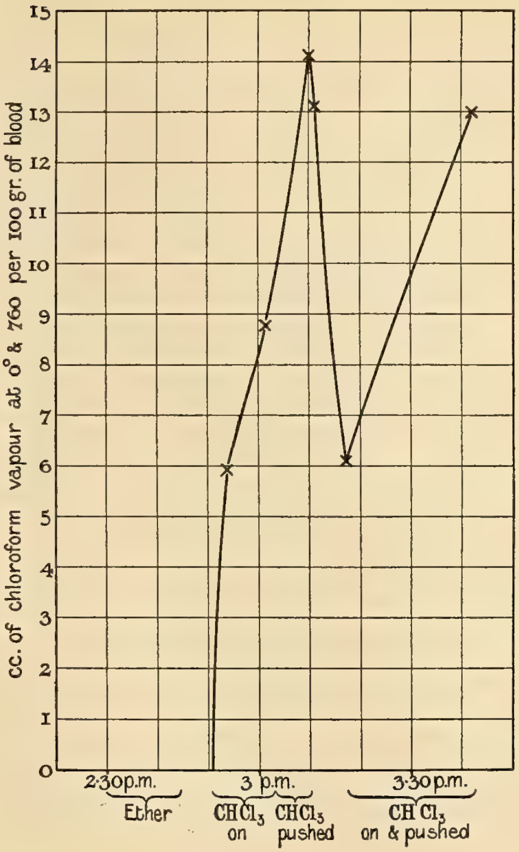
Experiment 10.—For the purpose of this experiment a dog weighing 10 kilogrammes was selected, and chloroform was administered by means of a Woulff's bottle, the temperature at the time being rather high—viz., 24° C., so that the percentage of chloroform in the air inhaled was probably somewhat higher than in those other experiments in which a Woulff's bottle was made use of. Blood samples were taken from the left carotid. The control sample was taken as usual under light ether anæsthesia, but in this case an extra sample of 10 c.c. was also withdrawn for another purpose. Chloroform was then administered, and samples of blood were collected when the reflexes had just gone and when the animal was deeply anæsthetised. At the latter stage an extra 10-c.c. sample was also taken for another purpose. The anæsthesia was then pushed by lowering the air inlet tube to the surface of the chloroform, and a sample of blood withdrawn when respiration ceased. The animal was now allowed to recover naturally, and samples were taken when breathing commenced regularly and when reflexes began to reappear. At the latter stage chloroform was again administered until the animal died, when a sample of blood was taken from the left auricle of the heart and analysed. The results of this experiment are recorded in Table X and Curve F′. As in the case of cats, the colour of the blood followed closely the percentage of chloroform in the blood.

Experiment 11.—In this case a very vigorous dog, weighing 10·5 kilogrammes, was selected, and the blood was withdrawn from the right carotid. The chloroform was administered from bags filled with mixtures of chloroform and air of known composition by the Dubois apparatus. The control sample of blood was taken under light ether anæsthesia, and at the same time a 10-c.c. sample for another purpose. Chloroform of 2-per-cent. strength was then administered, and duplicate samples taken when the reflexes had just gone. Ten minutes later another sample of blood was taken. At this stage the strength of the anæsthetic was increased to 3 per cent., and a sample of blood withdrawn eight minutes later. The strength of the chloroform was then increased to 4·5 per cent., and nine minutes later a duplicate sample of blood was withdrawn. Fourteen minutes later another sample was collected, and the chloroform was cut off. At this stage respiration *had not ceased*, but

Table X.—Dog. Weight, 10 kilogrammes. The chloroform was administered by means of a Woulff's bottle. Laboratory very warm, about 24° C. Left carotid tapped.

These figures were obtained by a less exact method than the others.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Differences from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
P.M.									
2.30	Ether started.								
2.45	,, ...	4 ·7500	0 ·0532	0 ·2761	—	—	—	Medium shade	Sample of 10 c.c. taken extra
2.47	Chloroform on.								
2.49	,, off.								
2.51	Chloroform on	—	—	—	—	—	—	—	Respiration 67 per min.; reflexes just gone
2.54	,, ...	4 ·8576	0 ·0599	0 ·3040	0 ·0279	0 ·0313	5 ·87	Rather darker	Respiration 65; blood darker than previous sample
3.1	,, ...	3 ·7845	0 ·0487	0 ·3179	0 ·0418	0 ·0470	8 ·82	Darker	Extra sample 10 c.c. taken
3.4	Chloroform pushed	—	—	—	—	—	—	—	Respiration 17; weak
3.10	,, ,,	3 ·7673	0 ·0523	0 ·3430	0 ·067	0 ·075	14 ·1	Very dark	Breathing stopped, after gasping. Allowed to recover with air
3.10½	$CHCl_3$ off.								
3.11	....	4 ·4874	0 ·0616	0 ·3384	0 ·0623	0 ·0699	13 ·1	Very dark	
3.17	$CHCl_3$ off	5 ·2033	0 ·0644	0 ·3051	0 ·0290	0 ·0325	6 ·1	Bright	Reflexes just appearing
3.17½	$CHCl_3$ on and pushed	—	—	—	—	—	—	—	Respiration 70
3.42	,, ,,	5 ·0958	0 ·0698	0 ·3377	0 ·0616	0 ·0691	12 ·98	Very dark	Animal died; sample taken from heart



F.

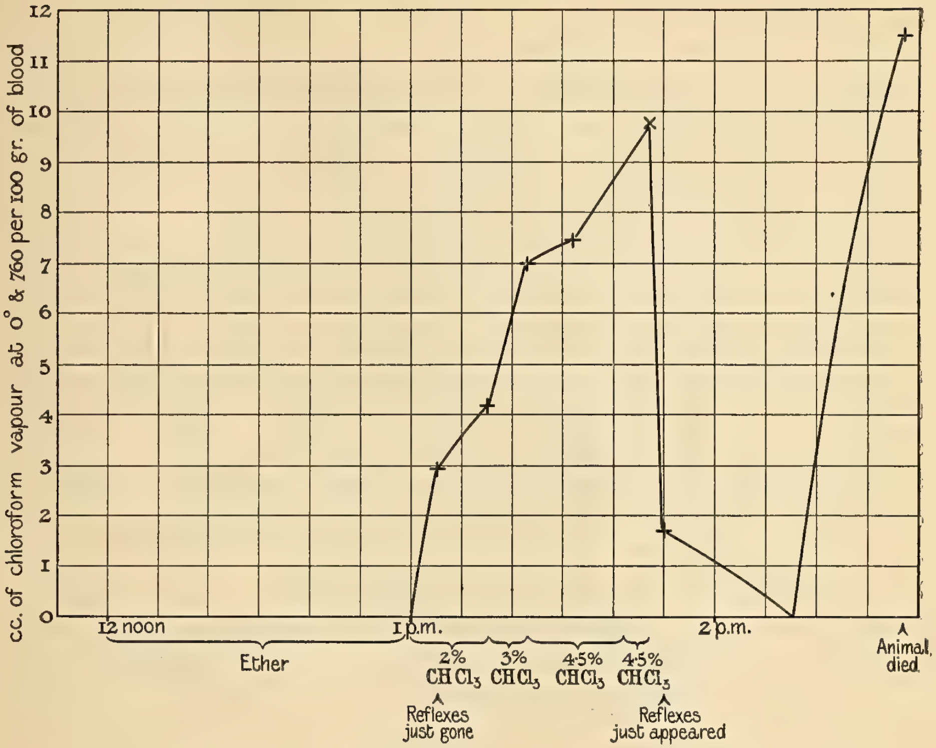
Constructed from Experiment 10. Samples of arterial blood (+).

was slow and shallow. During the later stages of the anæsthesia alternate twitchings of the paws were noticed, which, however, ceased as the asphyxiation point was approached. A sample of blood was collected when the reflexes again began to be quite evident, and to prevent the animal completely reviving a little ether was administered. Twenty-eight minutes after the chloroform had been cut off a sample of blood was collected, the analysis of which showed that the chloroform had been completely eliminated from the blood, and that the chlorine content had returned to normal. Chloroform was now administered rapidly by means of a Woulff's bottle, and a sample of the blood was collected after respiration ceased and as the animal was dying.

The results of the experiment are given in Table XI and Curve G.

Table XI.—Dog. Weight, 10·5 kilogrammes. Right carotid tapped. Chloroform given by Dubois.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Per-centage of chlorine.	Difference from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
12 noon	Ether began.								
P.M. 12.55	Very light ether	4·8512	0·058	0·2947	—	—	—	Bright	Reflexes visible; 10 c.c. blood taken for another purpose
1.0	Chloroform on, 2 per cent.								
1.4	" "	5·3664	0·0672	0·3087	0·0140	0·0157	2·95	Fairly bright......	Reflexes just gone; 10 c.c. blood taken
1.15	" "	4·7210	0·0602	0·3143	0·0196	0·022	4·14	Scarcely darker	
1.15½	3 per cent. $CHCl_3$ on.								
1.23	" " ...	6·0238	0·0801	0·3278	0·0331	0·0372	6·98	Darker	
1.23½	4·5 per cent. $CHCl_3$ on.								
1.32	" "	4·6429	0·0622	0·3302	0·0355	0·0399	7·49	Very dark	Breathing slow and shallow, but regular; muscular movements of tail began; 10 c.c. extra blood taken
1.37	" "	—	—	—	—	—	—	—	Alternate twitching of fore paws began
1.41	" "	—	—	—	—	—	—	—	These movements more marked
1.43	" "	—	—	—	—	—	—	—	Respiration very shallow
1.46	" "	5·6088	0·0776	0·341	0·0463	0·0521	9·77	Very dark indeed	Animal still breathing
1.46½	Chloroform off	—	—	—	—	—	—	—	Began to breathe rapidly; tail movements ceased
1.48	"								
1.50	"	4·5619	0·056	0·3026	0·0079	0·0089	1·66	Very bright	Reflexes began to be quite evident
1.52	Slight ether on.								
2.0	Slight ether on	—	—	—	—	—	—	—	Respiration quite regular and satisfactory
2.15	"	7·0214	0·0832	0·2921	−0·0026				
2.25	$CHCl_3$ on, using Woulff's bottle, with air bubbling through $CHCl_3$.								
2.37	" "	5·605	0·0794	0·3492	0·0545	0·0612	11·48	Very deep indeed	Animal dying



G.

Constructed from Experiment 11. Samples of arterial blood (+).

General Conclusions.

No previous observations on cats are available for reference; all the observers whose work has been considered in the earlier part of this paper carried out their investigations exclusively on dogs.

It is difficult in any case to ascertain the exact moment at which an animal is really anæsthetised. In our experiments we have taken the moment of the disappearance of both conjunctival reflexes as a fixed point, though the determination of this presents some difficulties, since the reflex may disappear from one eye a minute earlier than in the other. The cessation of the tail-reflexes may sometimes precede, sometimes succeed that of the conjunctiva. The times when samples of blood were taken in our experiments at this stage of anæsthesia slightly differ; for example, blood was withdrawn sometimes when the conjunctival reflexes definitely disappeared, sometimes when they definitely reappeared, sometimes at a point when they had almost vanished or had returned in one eye.

Combining the results of the experiments described, which form only a portion of a much larger number of observations, some of which were incomplete and only partly serviceable from the inevitable bursting of tubes, which may occur in carrying out a series of Carius' determinations for chlorine, the results of our experiments show that the amount of chloroform in arterial blood of cats at the stage of disappearance of conjunctival reflexes varies between 14 and 27·6 milligrammes per 100 grammes of blood. This amount varies with the individual animal, a fact noticed by others who have worked with dogs. This probably depends on the condition of the animal in the widest sense of the term; thus some were male, some female, and we were also in many cases ignorant whether the animals had been recently fed or the opposite. The ages of the animals were also unknown. Reference to the tables shows that body-weight is without influence on the percentage of chloroform in the blood necessary to produce anæsthesia.

An examination of Curves A, B, C, and D, shows that the rate of induction of anæsthesia varies for different individuals. This does not appear to be altogether dependent upon the percentage of chloroform in the inspired air, but is a feature peculiar to each individual animal. It can be seen from Curve D that the two upward slopes of the curve are closely similar in form in the same animal. In this curve, constructed from an experiment during which a constant percentage of chloroform was inhaled, the lethal point is raised during the second anæsthetisation—in fact, all the corresponding points of the second slope are higher than for the first. The explanation of this, which is a feature we have often noticed, might be that the first anæsthetisation is assisted by some ether which still remains in the animal. But this supposition is not confirmed by a consideration of Curves B and C. We are inclined to attribute the shifting-up of the second part of the curve and the raised lethal point to the loss of blood taken during the course of the experiment, as more blood was abstracted from the animal in Curve D than in experiments Curves B and C. The effect of removal of blood markedly alters the lethal dose in blood, a fact made quite evident in Curve E. However, at this stage of our work we do not propose to offer the suggestion that loss of blood is the only factor which raises the lethal dose, as we are still engaged in experiments devised to ascertain the progress of chloroform anæsthesia in animals under varied physiological conditions.

The lethal dose which arrests respiration in cats is also variable, and averages about 40 milligrammes per 100 grammes of blood, and it will be seen from the Tables that a narrow margin exists between the weight of chloroform in blood at the moment of anæsthesia (loss of conjunctival reflexes) and cessation of respiration. The lethal dose of Curves B and C is slightly higher in the second anæsthetisation than in the first

We are inclined to attribute this to slight excess of chloroform in the inhaled air.

After anæsthesia the chloroform is eliminated with extreme rapidity. The rate of elimination varies in different animals, but the rate of disappearance of chloroform is far more constant than the rate of assumption.

In the two observations quoted for dogs the results are not quite similar in character to those observed in cats, for the lethal dose and the quantity of chloroform required to produce various stages of anæsthesia are somewhat higher. The rapidity of elimination of chloroform appears to be even greater than in the case of cats. The figures we have obtained for dogs are in fair agreement with those of the French observers. Especially is this the case for the lethal dose, but the difference noticed for the anæsthetic dose depends probably on the criterion of anæsthesia adopted. The mean value in the arterial blood of dogs is given in following Table, though it must be pointed out that they used much heavier animals than those at our disposal.

Table XII.

	Tissot.	Nicloux.	Our experiments, reflexes just gone.
	milligrammes.	milligrammes.	milligrammes.
Anæsthesia	34—40	50	16—31
Lethal dose.........	59—105	69—73	61—69

II.—*Experiments to ascertain how the Chloroform distributes itself between the Corpuscles and the Plasma.*

In the experiments on this subject the animals were anæsthetised and the samples of blood were collected in exactly the same way as in the series already described. The samples were in most experiments mixed with a saturated solution of sodium oxalate, in the proportion of 0·5 c.c. oxalate solution to 10 c.c. of blood, to prevent clotting. They were then centrifugalised and the plasma separated from the red sediment by means of a fine pipette.

In other experiments 10 c.c. samples of blood were withdrawn from the animal, mixed with the oxalate and divided into two equal volumes before centrifugalisation. In a few experiments the blood was cooled by ice and centrifugalised in an ice jacket without the addition of oxalate. Following this plan it was found very difficult to get a satisfactory separation of the red corpuscles before clotting took place. We found that this could be effected much more easily in the case of cat's blood than in the case of dog's. The samples of corpusles and plasma were analysed in the same way as before.

With the centrifugal machines at our command we found it very difficult to get exactly equal ratios of separation of red matter and plasma in the control samples taken under ether and the samples taken under chloroform. As the percentage of natural chlorine in the red corpuscles is very different from that in the plasma it is obviously impossible to calculate with any degree of accuracy from the experimental results the exact distribution of chloroform between the corpuscles and plasma, unless the ratios of separation in the control and chloroform samples are sensibly equal. With very great care we succeeded in getting a sufficiently equal separation in a few experiments to permit of this calculation being performed with accuracy. In the other experiments, though the calculation could not be made, the results obtained indicated clearly that the chloroform associated itself with the corpuscles rather than the plasma.

Experiment 12.—In this experiment a cat was anæsthetised in the way described, using a Woulff's bottle. Samples of blood were collected under ether, and also when the animal was very deeply under the influence of chloroform. Clotting was prevented by oxalate. Unfortunately the Carius tubes containing the corpuscles exploded, but the percentages of chlorine found in the control sample and the chloroform sample were respectively 0·3819 and 0·3895. This was one of our earlier experiments, and the silver chloride was separated by filtration through paper and weighed in the usual manner instead of by the method described at the beginning of this paper; the results are, therefore, approximate, and we give them for what they are worth. They show, however, that little, if any, of the chloroform administered was in the plasma.

Experiment 13.—For the purpose of this experiment a cat weighing 2·8 kilogrammes was taken, the chloroform being administered by means of a Woulff's bottle, and the blood collected when the animal was deeply under. The samples of blood were centrifugalised in ice without the addition of oxalate. The ratios of separation in the control and chloroform samples were not, however, equal. The chlorine was estimated by the method used in the previous experiment, so that we do not regard the analysis as possessing the highest degree of accuracy.

The following Table XIII (p. 447) gives the result obtained.

Whereas the percentage of chlorine in the corpuscles shows an increase of 0·056, that in the plasma is only 0·005.

Experiment 14.—In this experiment a dog was anæsthetised with ether and a control sample of blood taken. Chloroform was then administered by

Table XIII.

	Weight of corpuscles.	Weight of plasma.	Weight of silver chloride from corpuscles.	Weight of silver chloride from plasma.	Percentage of chlorine.	
					In corpuscles.	In plasma.
Control expt., under ether	2·7095	2·8975	0·0342	0·0496	0·315	0·423
Expt., under chloroform	1·7798	3·4678	0·0268	0·0587	0·371	0·428

means of a Woulff's bottle, and three samples of blood collected at various stages of anæsthesia up to the asphyxiation point. An attempt was made to centrifugalise the blood simply cooled in ice without the addition of oxalate, but all the samples clotted. They were, therefore, allowed to stand until contraction took place, when they were again centrifugalised, and the corpuscles and serum analysed. The analysis was made by the same method as in Experiment 13. The ratios of separation of corpuscles and serum in the various samples were not equal, so that ratios of chloroform distribution could not be calculated. The results, however, indicate that practically no chloroform went into the plasma, except when the anæsthesia was pushed to an extreme point. The results are given in the following Table.

Table XIV.

	Weight of corpuscles.	Weight of silver in corpuscles.	Percentage of chlorine in corpuscles.	Weight of serum.	Weight of silver chloride in serum.	Percentage of chlorine in serum.
Control samples under ether	3·5942	0·0376	0·254	2·042	0·0321	0·3875
Under $CHCl_3$ when reflexes gone	3·3118	0·0378	0·282	2·258	0·0355	0·3876
Under $CHCl_3$ when breathing was very feeble	2·8333	0·0367	0·319	2·2084	0·0373	0·4173

Experiment 15.—In this experiment a cat weighing 3·1 kilogrammes was taken and anæsthetised with ether; 10 c.c. of blood were then withdrawn when the reflexes were scarcely marked, and mixed with $\frac{1}{2}$ c.c. of saturated sodium oxalate. The sample was then divided into two equal portions, each of which was weighed and centrifugalised. One sample was analysed and the other left for experiments which will be mentioned later; the animal was then allowed to recover partially, and chloroform was administered slowly by means of a Woulff's bottle. Samples were collected when the reflexes had just gone, and when respiration was on the point of ceasing. The samples were treated in the same way as the control sample. The analyses were made with the utmost care. The results are recorded in Table XV.

It will be noticed that the percentage of chlorine in the plasma remained constant, at any rate to within the probable errors of experiment. Calculation shows that 71 and 73 per cent. of the chloroform was taken up by the red corpuscles. Probably, however, a much higher proportion than this, perhaps the whole, was taken by the corpuscles, as the percentage of chlorine in the plasma remained the same. The discrepancy can, we think, be quite accounted for by the fact that the ratios of separation of corpuscles and plasma in the various samples were not quite equal.

Experiment 16.—In this experiment the samples of blood used were taken from the dog used in Experiment 10, chloroform being administered by the Dubois apparatus. The results are given in Table XVI.

Here again the bulk of the chloroform was absorbed by the red matter, and little went into the plasma. Calculation shows that at the point of the vanishing of the reflexes 64 per cent. attached itself to the corpuscular matter,

Table XV.—Cat.

Time.	Anæsthetic.	Weight of blood + 0·25 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.	Percentage of chlorine in corpuscles.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M.										
10.45	Ether began.									
11.20	Ether............	5·1975	0·0685	0·3249	—	—	—	1·9781	0·0184	0·2293
11.20	$CHCl_3$ on.									
11.34	,, ...	5·1823	0·0719	0·3420	0·01714	0·01924	3·6	1·9439	0·0209	0·2650
11.40	$CHCl_3$ pushed.									
11.46	,,	—	—	—	—	—	—	—	—	—
11.53	,,	—	—	—	—	—	—	—	—	—
11.54	,,	5·2267	0·0760	0·35844	0·0336	0·0377	7·07	1·8804	0·0237	0·3107

Percentage of chloroform taken by corpuscles
,, ,, ,, ,,

but in this case the calculation is vitiated even more than in the previous experiment by inequality in the ratios of separation, which were 1·034 in the control and 1·048 in the chloroformed sample later.

Experiment 17.—For the purpose of this experiment a cat weighing 3·5 kilogrammes was taken. A control sample of 10 c.c. was taken and mixed with ½ c.c. sodium-oxalate solution and centrifugalised. The cat after partial recovery was then slowly chloroformed, using bags filled with air containing approximately 2 per cent. of chloroform vapour, by means of the Dubois apparatus. A 10 c.c. sample of blood was collected when the animal was very deeply under the influence of the anæsthetic. The colour of the sample was very dark. It was treated exactly as in the case of the control. The samples, which were larger than in other experiments, were analysed with the utmost precaution. In this case we were fortunate enough to get a very good separation of corpuscles and plasma, and the ratios in the case of control and chloroform samples were very nearly equal.

The results are given in Table XVII. In this experiment the whole of the chloroform associated itself with the red corpuscles.

A number of experiments were performed, the results of which are not recorded, as, owing to accidents in the analysis, they did not form a complete series. The results obtained, however, were in accordance with those quoted.

Weight, 3·1 kilogrammes.

Percentage of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Remarks.
	As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.				
0·0873	—	—	—	3·2194	0·0501	0·3836	Sample taken when reflexes just present. Respiration 32 per min.
0·0994	0·0121	0·0136	2·56	3·2384	0·0510	0·3882	Sample taken when reflexes had just completely gone. Respiration 44 per min.
—	—	—	—	—	—	—	Respiration 28 per min.
—	—	—	—	—	—	—	Respiration 48, and very shallow
0·1118	0·0245	0·0275	5·17	3·3463	0·0523	0·3853	Respiration 20, and very shallow. Colour of blood very dark

71·1, at period of vanishing of reflexes.
73·1, at period of asphyxiation.

Table XVI.—Dog. Weight, 23 lbs.

Time.	Anæsthetic.	Weight of blood + 0·25 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
12 noon	Ether began.								
P.M. 12.55	Very light ether.........	4·8812	0·0544	0·27473	—	—	—	2·3997	0·0194
1.0	Chloroform on, 2 per cent.								
1.4	" "	5·0918	0·0601	0·29096	0·0162	0·0182	3·42	2·4859	0·0224
1.15	3 per cent. on.								
1.23	4·5 per cent. on.								
1.32	....	*	*	*	*	*	*	2·4743	0·0267

* These values could not be calculated, owing to
Percentage of chloroform taken by corpuscles

Table XVII.—Cat.

Time.	Anæsthetic.	Weight of blood + 0·5 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.	Percentage of chlorine in corpuscles.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
P.M. 3.0	Ether began.									
3.41	Ether on	10·1997	0·1340	0·32385	—	—	—	3·9132	0·0386	0·2431
3.42	Ether off.									
3.47	$CHCl_3$ on, 2 per cent.									
4.30	" "	10·0375	0·1400	0·34382	0·01997	0·0224	4·2	3·8050	0·0460	0·298

Percentage of total chlorine

Conclusions.—It would appear from these experiments that in cats the chloroform associates itself primarily with the red corpuscles, and never gets into the plasma unless the anæsthesia is pushed to an extreme point and the anæsthetic is rapidly administered. This conclusion also applies to dogs, but in this case the chloroform appears to enter the plasma somewhat more readily on pushing the anæsthetic. This conclusion is also in agreement with the results of Nicloux.

Chloroformed with Dubois apparatus.

Percentage of chlorine in corpuscles.	Percentages of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Remarks.
		As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.				
0·1993	0·09797	—	—	—	2·4815	0·0350	—	Reflexes just marked
0·2221	0·10844	0·0105	0·0118	2·2	2·6059	0·0377	0·3566	Reflexes gone
0·266	*	*	*	*	2·1102	0·0312	0·3636	Very deeply marked; blood very dark

a few drops of the total plasma having been lost.
at period of vanishing reflexes, 64·3.

Weight, 3·5 kilogrammes.

Percentage of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Colour.	Remarks.
	As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.					
0·09329	—	—	—	6·2865	0·0954	0·37409	Bright ……	Sample of 10 c.c. taken
0·11297	0·0197	0·0221	4·15	6·2325	0·0940	0·37179	Very dark	Sample of 10 c.c. taken

taken by corpuscles, 98·5.

The chloroform in the blood drawn from an anæsthetised animal appears to be very firmly held, as in none of the samples examined did we notice any marked smell of chloroform, and in some experiments blood allowed to stand over night suffered no diminution in chlorine.

In the hope of throwing some light on this question, duplicate samples of blood were withdrawn from an animal and centrifugalised. One series of samples were analysed directly and the other series after drying *in vacuo* over sulphuric acid, and finally heating for some hours in the steam oven.

Experiment 18.—In this experiment the samples were taken from the dog used in Experiment 10. The chlorine values obtained by direct analysis without drying are those quoted in Experiment 16.

In the following Table we give the percentages of chlorine found in the undried and dried samples of corpuscles and plasma:—

	Percentage of chlorine.					
	Corpuscles.			Plasma.		
	Undried.	Dried.	Difference.	Undried.	Dried.	Difference.
Control samples under ether	0·1993	0·1811	0·0182	0·3477	0·3284	0·0193
Sample under $CHCl_3$ when reflexes had gone	0·2221	0·1885	0·0336	0·3566	0·3223	0·0343
Sample under $CHCl_3$ when blood was very dark in colour	0·2660	0·2027	0·0633	0·3636	0·3188	0·0448

We were surprised to find that the control sample both of corpuscles and plasma lost chlorine on drying; this loss was more marked in the case of the plasma. An inspection of the Table, however, will show that the chloroform samples lost considerably more chlorine, and that the figures obtained after drying are almost identical in each set. All the chloroform therefore appeared to be eliminated on drying.

Experiment 19.—The results quoted in this experiment were obtained with the blood of the animal mentioned in Experiment 15, which was treated in the same manner as described in Experiment 18, but the heating was not so prolonged, and the temperature was not above 90°.

	Percentage of chlorine.					
	Corpuscles.			Plasma.		
	Undried.	Dried.	Difference.	Undried.	Dried.	Difference.
Control under ether ...	0·2293	0·2297	—	0·3836	0·3511	0·0325
$CHCl_3$ when reflexes gone	0·2650	0·2486	0·0164	0·3882	0·3518	0·0364
$CHCl_3$ very deeply under	0·3107	0·2854	0·0253	0·3852	lost	

In this case the corpuscle control suffered no loss, but that of the plasma was marked. Possibly this may be due to the fact that in this experiment the separation of the corpuscles and plasma was much more complete. In this case the chloroform was not completely eliminated from the corpuscles. The loss suffered by the control and chloroform plasma was practically the same, so that probably little or no chloroform was present.

Experiment 20.—In order to prove conclusively that the loss of chlorine noted in the control samples in the two previous experiments was really due to the evolution of free hydrochloric acid on heating, a cat was anæsthetised with ether, and bled. About 18 grammes of the blood were mixed with a saturated solution of sodium oxalate (neutral) in the proportion of 1 c.c. to 47 of blood and centrifugalised. 12·9 grammes of plasma and 4·9 grammes of corpuscles were obtained. The samples were then placed in desiccators and dried *in vacuo* over sulphuric acid at the temperature of the laboratory. In each desiccator was suspended a small tray of pure crystals of copper sulphate to absorb any hydrochloric acid that might be evolved. After the samples were dry the copper sulphate crystals in each case were dissolved in water and tested for chlorine. We were, however, unable to detect with certainty any trace of hydrochloric acid. It must be remembered, however, that the quantities evolved from the amounts of blood taken would be in any case exceedingly small. The dried samples of plasma and corpuscles were then placed in small flasks. Each flask was connected with a larger flask, partly filled with strong sulphuric acid, by means of a fairly wide tube containing crystals of pure copper sulphate, and the air was pumped from the apparatus. The flasks containing the dried matter were then heated for four or five hours on the water bath. At the end of the experiment, the copper sulphate crystals in each tube were dissolved in water and tested for chlorine.

Each solution was found to give a slight but quite definite precipitate with silver nitrate, which careful tests proved to be silver chloride. The precipitate appeared to be somewhat larger in the case of the plasma than in the case of the corpuscle. The precipitates were, however, too small to admit of being accurately weighed.

From these experiments it is therefore evident that dried blood loses traces of chlorine at temperatures between 80° and 100° C.

It is somewhat difficult to explain these rather unexpected results, but it may be that the loss of hydrochloric acid is due to the interaction of the sodium phosphate with the sodium chloride of the blood at the temperature of the experiment. This hypothesis is in accordance with the fact which we have before noticed that mixtures of common phosphate of soda and salt lose a slightly greater weight on drying than can be accounted for by loss of water, either by crystallisation or hydration.

In order to subject it to a still further test, 0·5 gramme of purified phosphate of soda was mixed with 10 grammes of pure salt—these being the approximate proportions in which these salts are stated to be present in blood—and the mixture was heated on the bath water for several hours in similar apparatus to that used in our experiments on blood. The copper sulphate was then dissolved in water and the chlorine present was estimated; 0·0805 gramme of silver chloride was obtained. Had the reaction taken place according to the following equation and gone to completion:—

$$Na_2HPO_4 + NaCl = Na_3PO_4 + HCl,$$

0·2 gramme of silver chloride should have been obtained. The experiment was repeated by a student in the laboratory of one of us with similar results.

These results appear to us to be of some interest in connection with Maly's theory as to the production of free hydrochloric acid in the organism.

We are engaged at present in further experiments based on the same plan of chlorine estimation, with a view to ascertaining the cause of anæsthesia under varied physiological conditions, some of the results of which we shall shortly be in a position to publish.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

On the Relation of the Liver Cells to the Blood-vessels and Lymphatics.

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(From the Physiological Laboratory of the University of Edinburgh.)

[Plates 22 and 23.]

The description by Schäfer (55) of a network of fine channels in the cells of the liver of the rabbit and cat which can be filled with injection material from the blood-vessels, and the confirmation of his observations in the livers of other animals as the result of our own experiments (26), have opened up several important questions concerning the minute anatomical structure of the liver. The presence of intracellular channels in the liver cells communicating with the blood-vessels is difficult to reconcile with the generally accepted views on the relations of the blood-vessels and lymphatics to the liver cells. Of late years several observers (Browicz (8), Schäfer (55)), have cast doubt on the presence of perivascular lymphatics in the liver lobules, and have suggested a direct supply of blood plasma from the vessels to the interior of the liver cells without interposition of lymph spaces. That the walls of the capillary blood-vessels of the liver possess a peculiar form of endothelial lining has been long recognised (Kupffer (37), Ranvier (50), and others). More recently Minot (45), from a study of the development of the liver vessels, has concluded that they are not true capillaries which have grown into the organ, but "sinusoids" which have been formed by a growth of the liver blastema into a large blood sinus, which, although having the appearance of capillaries, are actually spaces between the columns of liver cells lined by cells of an embryonic character.

To resolve the question of the relationship of the blood and lymph to the liver cells, we have in many kinds of animals injected the blood-vessels with carmine gelatine, and have, in dogs and cats, injected the large lymphatics of the liver with the same material. We have also injected the bile ducts in a number of animals and have further examined sections of liver stained by special methods. The results of our observations are recorded in this paper.

We are indebted to Professor Schäfer for help and advice in our work, and to Mr. Richard Muir for the care with which he has executed the accompanying drawings. The expenses of the research have been defrayed

by a grant from the Moray fund for the prosecution of research in the University of Edinburgh.

The Injection of the Liver Cells from the Blood-vessels: Method employed.

The injection material we have used in the majority of our experiments was carmine gelatine made up according to Carter's formula. The solutions of gelatine and ammoniacal carmine were filtered separately and very carefully, then mixed and rendered slightly, but distinctly, acid with acetic acid. With such an injection mass there is no staining of tissues, and no diffusion through the walls of ordinary capillaries. On some occasions we used Prussian blue gelatine and also watery Prussian blue, but we found both of these too diffusible; nor do they allow of such good fixation of the tissues as can be got by putting carmine gelatine injected preparations into cold formalin. We also tried thick suspensions of Chinese ink, and in one case hog's lard melted and filtered. Of all these, the carmine gelatine mass has given by far the best results.

The apparatus used for injection consisted of a large pressure bottle with rubber pump attached and another tube leading to a bottle containing the injection mass. A T-shaped junction on its course was led off to a mercury manometer, which indicated the pressure employed. The bottle containing the injection mass was immersed in a large bath of warm water; the mass flowed from it along a rubber tube to the cannula.

The injection was made as soon after death as possible. The animals were killed either by an overdose of chloroform or by coal gas. The thorax or abdomen was opened and the cannula tied to the aorta or the portal vein. The rubber tube and cannula were carefully filled with injection mass, to the complete exclusion of air bubbles, a side tube being adapted to the cannula to facilitate this. This side tube being then closed and the animal immersed in the water bath, the pressure was gradually raised by pumping air into the pressure bottle, the inferior vena cava having first been opened above the diaphragm to allow the free escape of blood. When the injection was made by the portal vein we ligatured the inferior vena cava below the liver to prevent any backward flow through the large veins to other parts of the abdomen. We did not, as a general rule, wash out the blood-vessels by the previous injection of salt solution, but when this was done we had a second bottle containing the saline solution attached by T-tubes to the same system; by opening and closing clips on the tubing we could inject either with carmine gelatine or with salt solution. The fluids injected, bottles, tubing, and animal were kept at body temperature by complete immersion in the water bath. The washing out of the blood-vessels by salt solution is unnecessary in the case of the liver, and as a rule we found that we got better injection preparations by using the carmine gelatine to drive out the blood, but the cells of the liver can be injected quite well after a preliminary injection of salt solution, and even after the injection of saline saturated with chloroform.

When the injection material was flowing freely from the opened inferior vena cava the portal vein was ligatured. In some experiments we allowed the injection material to fill up and distend the liver by ligaturing the inferior vena cava above and below the diaphragm before tying the portal vein, in others we left the inferior vena cava unligatured and allowed free escape of the fluid all the time, so as to avoid any excess of pressure. In one animal, a dog, the portal vein and hepatic artery were ligatured, and the cannula was put into the aorta close to the heart, a pressure of 120 mm. Hg. being used. There was a free escape all the time from the inferior vena cava above the diaphragm, but, nevertheless, some of the injection mass passed backwards along the hepatic veins and reached the central parts of the lobules of the liver. Even under these circumstances the liver cells near the central vein of each lobule were copiously injected with the carmine gelatine. The pressures employed varied from 60 to 160 mm. of Hg when the injection was made from the aorta, and rarely exceeded 20 mm. of Hg when made from the portal vein.

We have also injected several frogs with carmine gelatine. In order to get a free flow of the gelatine through the vessels, each frog was pithed and then placed for half an hour in the warm bath (37° C.), at the end of which time it was sufficiently warmed to prevent solidification of the gelatine.

In all cases, after the injection was completed, the liver was removed if the animals were large; if small, the abdomen and thorax were freely opened, and the liver or the whole animal placed at once in 10-per-cent. solution of formalin with some ice added. When the gelatine had set the liver was removed, cut into pieces and put back into 10-per-cent. formalin. When thoroughly fixed, pieces from different parts of the organ were dehydrated with alcohol, cut in paraffin, and lightly stained with hæmatoxylin. If weak hæmatoxylin be used the carmine retains its colour while the nuclei of the liver cells give a good blue contrast. Deep staining must be avoided as it stains the gelatine and masks the carmine.

The use of two injection masses of different colour we found to be unsatisfactory. We tried Prussian blue mass followed by carmine gelatine in one dog, keeping up the first injection for some time so as to fill the lymphatics of the liver. Carmine gelatine was then run through to wash away the Prussian blue from the blood-vessels, and to fill them with red injection.

After fixation with alcohol, sections of the liver showed a great deal of staining with Prussian blue; the blood-vessels were imperfectly injected; some contained the first injection, some nothing but carmine mass; but in most the two were mixed. The first injection tends to adhere to the walls of the blood-vessels, and is taken up by Kupffer's cells; this also occurs frequently in poor injections where blood and injection mass mingle, and it is the walls of the vessels which are chiefly coloured by the pigment. The liver cells in the above experiment contained Prussian blue mass in some situations, and carmine gelatine in others. We tried two different injection masses in several other animals, but the results were unsatisfactory, partly on account of the manner in which the first injection mass clings to the walls of the vessels, and partly because the Prussian blue (which we used for a colour contrast) is diffusible.

In several experiments, after injection of carmine gelatine, we washed out the vessels with a large quantity of salt solution. Sections of such livers showed the vessels for the most part free from colour, but the liver cells containing carmine gelatine, which was also present in Kupffer's cells.

Previous Observations.

That it is possible to inject liver cells from the portal vein was shown by Asp (3) in 1873. Asp employed a solution of alcannin in turpentine oil, and injected the portal vein with a pressure not exceeding 30 mm. Hg. The alcannin finds its way into the liver cells, which present appearances similar to those produced in them by injecting the bile ducts. Asp specially noted that though the alcannin penetrates into the liver cell, both from bile ducts and capillaries, it does not pass through the liver cell, so that capillaries cannot be injected from the bile ducts, nor bile ducts from the capillaries. No particular notice appears to have been taken of this discovery, nor, apparently, did Asp himself attach importance to it.

It was noticed by Hüttenbrenner (31), and later by Rutimeyer (54), that cinnabar particles, when introduced into the circulating blood by the jugular vein, are soon found in the liver cells (among other places). Rutimeyer found them in the cells of the peripheral parts of the lobules an hour after injection in the dog, and more uniformly distributed if a longer time had elapsed between the injection and death. The same is true of the fat globules after intravenous injection of milk.

In 1895 J. W. and E. Hewat Fraser (20) drew attention to the presence of inter- and intra-cellular passages in the liver of the frog. They used both Hoyer's lead chromate gelatine and Carter's carmine gelatine, and injected from the bulbus aortæ at a pressure of from 1 to 4 inches of mercury.

They stated that the injection material penetrates between the liver cells and finds its way into them by one or more fine channels, reaching even into the nucleus and ending in knob-shaped dilations. Increase of injection pressure causes an accumulation of the injection mass in reservoirs or vacuoles in the cell protoplasm, but does not distend to any appreciable extent the fine passages leading to them from the blood-vessels. The brothers Fraser concluded that in the frog at least a system of fine plasmatic channels exists in the liver, too small to admit the corpuscles of the blood, but affording a direct means of communication between the blood plasma and the interior of the liver cells.

Nauwerck (46), after reading Fraser's paper, looked carefully over a series of preparations of injected human livers, and found the injection present in the liver cells, often in ring-shaped canals round the nucleus, but never penetrating into it. Nauwerck described two intra-cellular networks of fine channels, one system belonging to the bile ducts and revealed by special stains, the other pertaining to the blood-vessels.

Browicz (9), from a study of normal and pathological human livers, and from the result of the intravenous injection of solutions of hæmoglobin in the dog, came to the conclusion that there must exist in the liver cells special afferent nutritive channels or canaliculi. These channels convey plasma and even red blood corpuscles directly to the nuclei of the liver cells. The red blood corpuscles are broken down in the cells, and the hæmoglobin is stored in the nuclei and converted into bile pigment; a second set of fine intra-cellular channels conveys the bile as it is formed into the bile canaliculi. Browicz (8) doubted the presence of perivascular lymphatics round the liver capillaries, and believed in a communication between the blood capillaries and the cytoplasm and even the nuclei of the liver cells. He found red blood corpuscles in various stages of disintegration in the liver cells. If in the dog's liver hæmoglobin had been injected intravenously some hours before death, free hæmoglobin and crystals of hæmoglobin were present both in the cytoplasm and in the nuclei. Browicz did not see the intracellular canaliculi, but argued that they must be present, though exceedingly fine.

In 1901 Schäfer (55) described specimens of livers of rabbit and cat in which the liver cells everywhere contained networks of injection material—carmine gelatine—which had entered them from the blood-vessels. The livers had been injected from the portal vein, but no record of the pressure employed had been kept. Schäfer described the injection within the cells as "confined within sharply-defined, somewhat varicose, intercommunicating canaliculi, many of which are in the immediate neighbourhood of the nucleus or nuclei," but he did not observe any injection actually within the nuclei.

He described the injection as having the same intensity of colour within the cells as in the blood-vessels, and hence inferred that it had not passed into the cells by way of lymph spaces, or it would have become diluted with lymph. Nor had the injection passed into the cells after having been extravasated into the intercellular biliary canals, for the latter contain no trace of injection and were, indeed, completely invisible. This was also the case with the perivascular lymphatics of the lobules (if such vessels exist). In the substance of the lobules the injection was confined to the blood-vessels and to the intracellular channels. There was no diffusion of carmine, and the cell nuclei were wholly unstained. The injection material was also apparent, but of a fainter colour, *i.e.*, in a diluted condition, in the lymphatics accompanying the branches of the portal and hepatic veins. The connective tissue around these vessels and extending a short distance into the lobules was stained by carmine. Schäfer was unable to detect the existence of perivascular lymphatics in the liver lobules. His description of the injection appearances agrees very closely with what Browicz assumed might be found. Browicz, to whom Schäfer sent preparations, states in a later paper that " the injection appearances are nothing less than ideal."

In 1902 E. Holmgren (28) described channels in the liver cells of the hedgehog. Within these channels are what he terms " Trophospongien," viz., the intracellular processes of certain multipolar cells. Functional or metabolic changes inside the processes lead them to become more or less fluid in character, and give the cell in which they are embedded the appearance of possessing a network of canals. In a later paper Holmgren (29) criticises the views of Browicz and Schäfer. He states his belief that the plasmatic channels described by them are the same as his " Trophospongien," and as such are not in direct connection with the blood-vessels, but open into perivascular lymphatics. The " Trophospongien " are, according to his view, processes of Kupffer's cells which extend into the protoplasm of the neighbouring liver cells, and he considers this view the more probable because of the property Kupffer's cells possess of destroying red blood corpuscles, thereby constituting a kind of trophic element in connection with the liver cells. In yet another paper Holmgren (30), after repeating the statements he had already made, expresses the opinion that the star-shaped connective-tissue cells, described by Reinke in the liver, may with great probability be regarded as the origin of the " Trophospongien " of the liver cells.

With regard to the injected preparations described by Schäfer, one of which he had the opportunity of examining, Holmgren enunciates the confident opinion that the intracellular injection described is an artefact produced by too high pressure having been employed in making the injection, and believes that he can show that the injection material in these preparations has passed from the blood-vessels into the liver cells by way of the lymphatics.

Schäfer (56), in replying to Holmgren's criticisms, points out that although the pressures employed in injecting the preparations described are not known, the appearances cannot be artefacts, for the injection is uniformly present in the cells throughout the liver, except in the immediate neighbourhood of extravasation, where it is absent or imperfect. The appearances which Holmgren considered to indicate the injection of perivascular lymph spaces are merely clefts in the gelatine produced by shrinkage during the process of hardening by alcohol, a portion of the gelatine tending, under these circumstances, to adhere to the wall of the capillary. It is this layer which Holmgren has taken to represent the injection of a perivascular lymphatic.

Description of our Experiments: Results Obtained.

It was to settle the question as to what amount of pressure is required to inject the liver cells from the blood-vessels that we began our work, a preliminary note of which was published last year. We made a commencement by injecting rats with carmine gelatine from the aorta in the manner already described. The rat was selected partly because of its small size, and partly because Dr. H. K. Anderson, of Cambridge, had sent to Professor Schäfer last summer specimens of rat's liver in which the cells were beautifully injected with carmine gelatine.*

In all, 20 rats were injected from the aorta close to the heart, at pressures varying from 80 to 120 mm. Hg. The inferior vena cava was opened above the diaphragm, and in most cases remained open so as to allow a free escape of blood and injection mass. In others the inferior vena cava was ligatured after the injection mass had begun to flow out, and the injection pressure was maintained some minutes longer before tying the portal vein. In all these rats the liver cells contain carmine gelatine. Some of the best specimens were obtained with a pressure of 80 mm. Hg in the aorta, and a free escape from the inferior vena cava during the whole period of injection, which occupied about five minutes. This pressure cannot be called excessive. The flow from the inferior vena cava was quite free all the time, and the pressure in the vessels of the liver must have been very low. Fig. 4 is from a specimen injected at this low pressure. Increase of pressure distends the blood-vessels and increases the amount of injection mass in the liver cells—not by the appearance of more canals, but by the widening of vacuole-like spaces on their course. The intracellular channels of the rat's liver appear, therefore, to be very easily injected; in fact, it would seem difficult to avoid injecting them.

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* Dr. Anderson informs us that the rat was injected from the aorta, and that during the injection there was a free escape from cut intercostal arteries; therefore the pressure in the portal vein could not have been excessive.

We are able to confirm the observations of the brothers Fraser on the frog; a pressure of 20 mm. Hg at the bulbus aortæ injects the cells of the liver so as to show fine channels ending in vacuole-like dilatations. We are unable to find any injection in the nuclei. Carmine gelatine is not, however, a very suitable injection for cold-blooded animals.

Four rabbits were injected; all show the fine intracellular channels described by Schäfer. Three were injected from the aorta with pressures varying from 100 to 120 mm. Hg; one from the portal vein with 30 mm. pressure. But in this latter experiment there was considerable leakage from rupture of one of the large branches of the portal vein, and the pressure in the liver must therefore have been less than that recorded by the manometer.

Two guinea-pigs were injected with aortic pressures of 100 mm. and 120 mm. Hg respectively. The injection mass did not run well in the first experiment, but the livers of both animals show intracellular injection.

One hedgehog, injected from the aorta at a pressure of 120 mm. Hg, shows the intracellular injection appearances; there was a free flow of injection mass from the inferior vena cava the whole time. In the only other hedgehog tried the injection fluid did not flow properly, and the injection failed, the vessels of the liver being incompletely filled. The injection mass employed in this case had been made up a considerable time, and we have found by experience that the best results are obtained with freshly prepared material.

In the dog, every injection shows the intracellular channels. In one animal, a young one, a pressure of 100 mm. in the aorta gave injection appearances throughout the cells of the liver. In another the injection was made by the portal vein, the inferior vena cava having been opened in the thorax and ligatured in the abdomen below the liver. Three hundred cubic centimetres of Ringer's solution, saturated with chloroform, was first perfused at a pressure of 20 mm. Hg; the carmine gelatine was then allowed to run in, the inferior vena cava next ligatured, and a pressure of 20 mm. Hg maintained during 10 minutes. Many of the liver cells contain carmine gelatine, which has the appearance of being accumulated chiefly in rounded masses as if in distended vacuoles.

In one of the injections from the aorta, as already mentioned, the portal vein and hepatic artery had been tied prior to the experiment, and the injection mass passed backwards from the inferior vena cava along the hepatic and sub-lobular veins, and reached the inner parts of the lobules, filling the cells copiously with injection. In this case the pressure in the hepatic veins must have been very low, because there was a free escape all the time from the inferior vena cava in the thorax.

The portal vein of another dog was injected for one hour with Prussian blue gelatine at a pressure of 40 mm. Hg, the object of the experiment being to fill the lymphatics of the liver with blue injection mass. Carmine gelatine was next run through until it escaped from the inferior vena cava, then the large vessels were ligatured and the liver placed in cold alcohol. Sections show Prussian blue gelatine in some cells and carmine gelatine in others. The Prussian blue is, however, very faint and diffuse in those lobules which are well injected with the carmine mass, but of a deep colour in the connective tissue of Glisson's capsule.

Another dog was injected with melted and filtered hog's lard through the portal vein at a pressure of 40 mm. Hg, a piece of liver having been first removed as a control. The cells of the injected liver are full of fat globules, as shown by staining with Sudan III and osmic acid.

In the cat a pressure of 100 mm. Hg in the aorta with free flow from the inferior vena cava gives a plentiful injection of the cells throughout the liver. We injected seven cats, with positive results in every case. A pressure of 20 mm. Hg in the portal vein is sufficient to give very good injection of the cells. In one cat 600 c.c. of Ringer solution, saturated with chloroform, was perfused through the liver from the portal vein. Carmine gelatine was then injected at a pressure of 40 mm. Hg; the injection appears in the liver cells. Chinese ink rubbed up with salt solution was also employed in one animal. Some of the smaller particles were found in the liver cells, but most of the particles were too coarse.

One ferret was injected from the aorta with a pressure of 80 mm. Hg, the inferior vena cava being open the whole time. The liver cells show intracellular injection to a marked degree.

The monkey presented unexpected difficulty. One only (*Macacus rhesus*) was successfully injected from the portal vein with a pressure of 60 mm. Hg, the inferior vena cava being open. The liver of this monkey shows intracellular injection, but not uniformly. The intracellular channels seem to be much finer than those of other animals. In some cells there are rounded accumulations of injection material, as though lying in distended vacuoles (fig. 6). Two other monkeys injected from the aorta, at pressures of 120 and 130 mm. of Hg respectively, show intracellular injection, but not well marked. Others failed to show the intracellular channels. The cells of the monkey's liver appear, therefore, to be injected less readily than those of the other animals investigated, and for their injection comparatively high pressures were necessary. But whether this result is due to the fine calibre of the channels, or to some unknown accidental circumstance, we are unable to say.

Three birds, a common fowl and two pigeons, were injected from the right aortic arch at pressures of 100 and 130 mm. Hg. Many of the liver cells contain fine threads of carmine gelatine, with occasional rounded or irregular accumulations. In these birds, as in the monkey, the intracellular passages are very fine (fig. 9).

From what has been stated about the pressures of the injections employed, it will be seen that they are not excessive, and many of them might quite well be exceeded by that of the blood during life. When the inferior vena cava was ligatured before the injection pressure was cut off, the pressure in the liver vessels must have risen considerably, but even in these cases, when the injection was made from the aorta, the mass, with the exception of that entering by the hepatic artery, has already traversed one set of capillaries before it reaches the liver. As has already been insisted on, some of the best injections were made with the inferior vena cava open. In the rat an aortic pressure of 80 mm. Hg, with free escape of the injection mass from the inferior vena cava, yielded one of the best of our preparations (fig. 4), the cells being injected uniformly throughout the organ. In one cat a pressure of 20 mm. Hg in the portal vein, with free escape from the inferior vena cava, also yielded a typical injection of the cells. In a dog, with hepatic artery and portal vein tied, there was sufficient backward flow to inject the cells at the central parts of the lobules, although the inferior vena cava was open in the thorax.

The appearance of injection material in the liver cells cannot therefore be ascribed to excess of pressure in the blood-vessels. The character of the injection in the cells, too, as was pointed out by Schäfer, is against any such supposition. In many places there are definite fine channels continuous with the lumen of the blood-vessel, forming a network within the cell. Dilatations on the network are often seen. These are probably vacuoles in the cytoplasm, and may be more distended with increase of pressure, but the typical network is best seen when a moderate pressure only has been employed.

The presence of injection within the cells is not the result of vital activity of the cell protoplasm. The circumstances of its occurrence are against this supposition, as well as the fact that the cells can be injected a considerable time after the death of the animal. Perfusion of large quantities of chloroform saline does not prevent the subsequent injection of the cells; and in one cat, perfusion of 300 c.c. of a 2-per-cent. solution of cyanide of potassium was followed by a successful result, although in this experiment the liver cells were considerably altered and the nuclei shrunken.

The appearances are against simple filtration from the blood-vessels into the cells. The colour of the carmine mass is of the same tint within the cells as in the blood spaces, and in many places a direct connection between the two can be made out. There is, besides, no diffusion of carmine and no staining of nuclei by it.

The question of the relation of the lymphatics to the injection is an important one. The injection certainly passes readily into the lymphatics and appears in the large trunks of the portal spaces. In these situations the injection mass contained in the large trunks is of a lighter colour and is obviously diluted with lymph, but the injection mass within the lobules shows no such appearance of dilution, nor, indeed, are any spaces visible but the blood spaces occupied by the injection. The mass in these is usually in close contact with the liver cells, and where spaces exist between the two they are clearly the result of shrinkage of the gelatine. If perivascular lymphatics exist in the lobules they must be filled with the injection mass, if it is through them that the liver cells become injected. We can make out no appearances suggestive of such a path for the injection. The injection is inside the blood-vessels and inside the liver cells; nowhere else within the lobule.

The relation of Kupffer's cells to the injection is an interesting one and will be dealt with more fully later. In a complete injection these cells are more or less hidden by the carmine gelatine, but in an imperfect injection, where comparatively little colouring matter is mixed with the blood, it is seen that Kupffer's cells are frequently coloured as though they had picked up the carmine from the vessel contents. (The presence of colouring matter on either side of the nucleus of a Kupffer's cell gives in such cases an appearance which might be mistaken for carmine gelatine on either side of the vessel wall.) When the blood-vessels are washed out after an injection of carmine gelatine, the injection mass often adheres to the vessel wall closely applied to the liver cells, and is especially noticeable at the Kupffer's cells. The cytoplasm of these cells is usually injected, but not the nucleus. The injection within their cytoplasm seems to be uniform and not confined to channels as within the liver cells, but the cytoplasm of Kupffer's cells is so small in amount that it is difficult to determine what has really happened, whether injection, infiltration, or absorption. The latter is possible and seems probable where the injection mass is small in amount and mixed with the blood.

We have seen no evidence in support of Holmgren's theory of Kupffer's cells sending processes into the liver cells to form "Trophospongien." The injection in the liver cells bears no apparent relation to Kupffer's cells; moreover, the latter are placed at comparatively wide intervals from one another, and it is inconceivable that they should send processes to all the intermediately situated liver cells.

A perfectly satisfactory conclusion with respect to the lymphatics can only be arrived at by the actual injection of the lymphatics of the liver. We shall revert to the subject again when dealing with the injection of the lymphatics.

In none of the specimens is there any indication of the injection having burst into the bile ducts or capillaries and so entered the liver cells. The characteristic network of the bile capillaries is not seen in any of our injected specimens, and there is no injection inside any of the issuing bile ducts, nor are the epithelial cells lining the ducts injected. After the injection mass has entered the liver cells it is difficult to wash it out by perfusion of the vessels with salt solution.

We have little to add to the description of the intracellular channels already given by Schäfer in the rabbit and cat liver. They form an irregular network in the liver cell. This network may be in direct communication at more than one point with the blood spaces. As a rule, the channels leading into the cell are fine—far too fine to admit red blood corpuscles; sometimes, however, a comparatively wide opening is seen. A ring-shaped channel is sometimes seen round the nucleus, as described by the Frasers in the frog, and by Nauwerck in the human liver. The injection frequently has the appearance of rounded or irregularly shaped accumulations, as if in vacuoles of the cytoplasm; a cell may contain many such clumps, and connections between them are not always apparent.

The injection is often seen in close contact with the nucleus, or both nuclei where two are present. As a rule it does not penetrate into the nuclei, but in the rat we have found it inside the nuclear membrane; when this is the case, it appears diffused throughout, and not lying in special intranuclear channels. The intracellular channels vary to some extent in the livers of animals of different species, but their general features are the same. The network arrangement is best seen in the rat and the rabbit. They are larger and more moniliform in the cat, still more so in the dog. In the monkey, as already intimated, they are very fine and less readily injected; and in the few birds we have examined they are also very fine.

We have not extended our observations to reptiles and fishes.

Our attention has been drawn by Mr. Richard Muir, of the Pathological

Department of the University, to a preparation of human liver from a case of so-called chloroform poisoning. There is extreme fatty degeneration (?) of the liver cells, and fat emboli in the blood-vessels throughout the body. In the liver, many of the vessels are filled with fat in continuous lines, and here and there are distinct connections between the fat in the cells and the fat in the vessels. The appearance is, indeed, very similar to the injection appearances recorded. The preparation was stained with osmic acid (fig. 10).

In examining sections of the dog's liver (uninjected) stained with eosine and methylene blue we have frequently seen crystals in the nuclei of the liver cells. The crystals are prismatic in shape and vary in length. Some are short and cause no distension of the nuclear membrane, while others are as much as three times the diameter of an average liver cell nucleus, and the nuclear membrane has the appearance of being stretched by the crystal (figs. 1 and 2). We have never seen these crystals in any other situation than inside the nuclei of liver cells, and they seem to occur with equal frequency in the lightly staining and in the darkly staining nuclei. We have seen them in the dog's liver in specimens from five different animals. Most of the preparations were fixed in 10 per cent. formol, but one specimen was fixed several years ago in corrosive sublimate for class purposes; sections of it show numerous crystals. Not more than one crystal is found in a nucleus. There is little nuclear network in the liver cells of the dog, but a nucleolus is present and is in the crystal-holding nuclei always situated close to the nuclear membrane on one side of and immediately opposite the middle of the crystal. The crystals stain with eosine rather more deeply than the nucleoli and red blood corpuscles. They are of prismatic form, with sharp edges, and closely resemble crystals of hæmoglobin. In some nuclei, irregular or rounded masses similarly staining appear. In the cytoplasm of some cells red blood corpuscles are present, some of which are unaltered and others more or less disintegrated. The crystals are probably composed of hæmoglobin or, at any rate, of some derivative of the blood pigment. That they are formed during life is obvious from the enlarged size of the nuclei which contain them and the adaptation of the shape of the nuclei to the size and shape of the contained crystal.

Similar crystals were described by Browicz in 1899. Browicz found them in the dog's liver after the intravenous injection of a solution of Merck's hæmoglobin. He also described the breaking down of red blood corpuscles in the liver cells and storage of hæmoglobin in the nuclei. The presence of hæmoglobin in the liver cell nuclei was one of the chief arguments advanced by Browicz in favour of a very intimate relationship between the circulating blood and the interior of the liver cells.

In the dog's liver there is, then, as Browicz originally stated, good evidence that red blood corpuscles pass into the liver cells and are broken down there. Hæmoglobin readily crystallises in the dog, which may account for its presence in a crystalline form in the liver cells of this animal. Further search may show similar crystals in the liver cells of other animals, although we have ourselves failed to find them, nor have we succeeded in finding them in the dog in unfixed and unstained sections.

The Lymphatics of the Liver. (*Observations of Previous Inquirers.*)

The lymphatics of the liver were investigated by Mascagni, Cruickshank, Lauth, Arnold and Sappey. As a result of the work of these and other anatomists arose the classical division of the liver lymphatics into a superficial and a deep set. The method employed by the older investigators was that of injection with quicksilver, whereby the vessels were distended and made clearly visible.

Kiernan (33), in 1833, stated that when the bile ducts are injected with fluid the injection material frequently passes into the lymphatics, and that even the injection of the portal vein or hepatic artery may be followed by a like result. Beale (4), in 1859, made use of this fact to inject the lymphatics of the ox's liver. He perfused water through the portal vein at a moderately high pressure until the main lymphatic trunks were distended. A cannula was then tied into one trunk and the liver subjected to pressure to squeeze out the fluid. When the lymphatics were thus emptied an injection of coloured material was made through the cannula; sections of the liver were subsequently cut and examined. Beale described lymph vessels on the surface of the liver and in the portal spaces, and thought they might occur inside the lobules, but he had not sufficient evidence to make a positive statement on this point. Teichmann (59), in 1861, described the lymphatics of the human liver. A superficial set forming an irregular network below the peritoneal coat passes by the ligamentum suspensorium to the diaphragm, and through it to join the thoracic duct. On the concave surface of the liver the superficial vessels unite near the gall bladder, some pass to the convex surface of the liver, some to the portal vein, and others sink into the substance of the liver to join the deep set. The latter vessels accompany and surround the branches of the portal vein, hepatic artery and bile ducts, and run between the lobules, but Teichmann could not follow them into the lobules. The injection mass frequently passed through the lobules as far as the central vein. Teichmann could not satisfy himself that it was in lymphatics. The deep set runs to lymphatic glands along the portal vein and then into the thoracic duct.

In 1864, Carter (13), employing carmine gelatine as an injection mass, came to the conclusion that there exists in the liver "a direct communication between the lymph vessels and those of the blood, and their distal as well as their proximal extremities, and in the former position through tubes of dimensions so small as to preclude the possibility of the blood corpuscles entering them." Carter also noted that injection of the portal vein of the liver with injection mass of one colour followed by injection mass of another colour results in a mixture of the two in the lymphatics. He found lymph vessels passing into the lobules and ending in nucleated fusiform cells, and the injection, he said, passes into these cells, which he regarded as the origin of the lymphatics.

In the same year, 1864, MacGillavry (42) published the results of his researches on the lymphatics of the liver. His observations have been widely accepted, and his paper is perhaps the one on the subject which is best known, although much of his work had been anticipated by Beale and by Carter. MacGillavry ligatured the lymphatic trunks of the liver in the portal fissure of a living dog. The lymph in such an experiment soon distends the vessels and brings about a natural injection. MacGillavry could not find the surface lymphatics described by Teichmann and earlier workers, and assumed that they are not a feature of the liver in the dog (and rabbit), but he noticed a large trunk on the gall bladder with numerous branches coming to it from the parenchyma of the liver. The main vessel on the gall bladder accompanies the cystic duct, and ends in a lymphatic gland near the duodenum. The deep lymphatics issue from every lobe of the liver, and follow the portal vein, appearing like strings of pearls, because of the numerous valves on their course.

In another series of experiments the lymphatics were injected with a cold watery solution of Prussian blue. To enable the injected fluid to overcome the resistance offered by the valves, MacGillavry steeped the liver for several hours in weak spirit prior to making the injection. (The alcohol causes the valves to shrink and so renders them inefficient.) A cannula was then inserted into one of the main trunks near the portal fissure, and injection fluid forced in. In some experiments the blood-vessels were subsequently injected from the portal vein with material of a different colour. The livers were hardened and sections cut. MacGillavry described the origin of the deep lymphatics from three different sources: (1) A tubular network surrounding the blood capillaries and stretching from the borders of each lobule to its central vein, and looking very like a network of injected capillaries. In transverse section of the blood capillaries of a lobule, the lymphatics surround each vessel in a ring-shaped manner, and the walls of the lymphatics are composed of fine connective-tissue fibrils on the one hand and liver cells and bile capillaries on the other; (2) lymph lacunæ lying in the interlobular connective tissue; (3) narrow anastomosing tubes of spindle-shaped appearance with much sharper outlines than are possessed by the ordinary lacunæ; these also lie in the connective tissue.

No lymphatics occur where there are no blood-vessels, and the latter are everywhere accompanied by perivascular lymphatics.

MacGillavry's results were not accepted by Hering (25), who also injected the lymphatics. Hering could find no evidence of a perivascular injection in the lobules of the rabbit's liver, and although he succeeded in producing in the human liver and in the dog's liver the appearances shown by MacGillavry, he expressed the belief that they are artificial spaces resulting from imperfect methods of injection and preparation. He pointed out that the previous soaking of the liver in spirit must produce shrinkage and alteration in other parts of the liver besides the valves of the lymphatics, and that the injection passes into clefts which do not exist during life, but are the result of *post-mortem* changes. In the rabbit's liver Hering found no trace of perivascular spaces in the lobules; the cells of the capillary wall are in direct contact with the liver cells, and bile capillaries do not come in contact with blood capillaries at any place as stated by MacGillavry. Hering thought it probable that MacGillavry had produced extravasation and rupture into the blood capillaries, and that subsequent injection of these with material of a different colour gave rise to the appearance of one injection surrounding the other in a ring-like manner. Hering also criticised the method of filling the lymphatics by injection of the bile ducts. He found that by careful injection of the bile ducts the injected material can be made to pass into the bile capillaries at the periphery of the lobules and enter the liver cells there, at first in small amount, but later in sufficient quantity to fill the cells entirely; rupture then takes place into the blood-vessels. If the blood-vessels be now injected with a differently coloured mass, sections of the liver will show the first injection material surrounding the second, and giving appearances which MacGillavry said could only be produced by the distension of perivascular lymphatics.

Irminger and Frey (32) injected the bile ducts with watery Prussian blue and described extravasations into the lymphatics, which they said looked like blood-vessels. They were able to obtain these results only in the rabbit's liver; they agreed with MacGillavry that there are perivascular lymphatics in the lobules.

Biesiadecki (7), in 1867, described spaces between the vessel walls and liver cells in human livers which had been the subjects of chronic venous congestion ; he considered these to be lymphatics. He made several attempts to inject them, but could not succeed in forcing injection mass past the valves of the main lymphatic trunks, and when he employed the method of injection by puncture the injection material flowed equally into blood-vessels and the spaces round them.

Kölliker (35) emphasised Hering's opinion that in the rabbit's liver, at all events, extravasation from the bile capillaries passes not into lymphatics, but into the blood-vessels.

Kisselew (34), in 1869, injected the liver of the dog and pig, using injection mass of one colour for the lymphatics and of another colour for the blood-vessels. He described perivascular lymphatics within the lobules with walls consisting of fine fibrillar material and endothelial cells. He also found lymphoid nodules in the substance of the pig's liver. He agreed with MacGillavry that the capillaries inside the lobules are surrounded by lymphatics.

Asp (3), in 1873, injected the bile ducts of the rabbit's liver with a solution of alcannin in turpentine oil and found that it passed from the bile capillaries into the liver cells. He also injected watery Prussian blue, and found it throughout the lobules outside the blood-vessels occupying spaces which he considered lymphatics.

Fleischl (19), in the following year, laid stress on the fact noted by Ludwig that after ligature of the common bile duct in the living dog the lymph issuing from the liver is tinged with bile, showing that the obstructed bile channels at some part of their course come into communication with the lymphatics. He tried injection of the bile ducts in the dog with alcannin dissolved in turpentine oil, but the experiments were not successful. The rabbit gave him better results, and Fleischl supported MacGillavry in believing in the existence of lymphatics within the liver lobules.

Wittich (62) came to the same conclusion after employing the method of *intra vitam* injection of sulphindigodate of soda in rabbits.

In 1875 Budge (12) described the results of a large number of injections he had made of the lymphatics of the liver in different animals. He states that asphalt dissolved in chloroform makes the best injection fluid for the lymphatics, and that it is advisable to inject the blood-vessels in addition with coloured gelatine. He also injected the lymphatics with a solution of nitrate of silver to determine whether they are lined with endothelial cells. Some of the injections were made by tying a cannula into one of the large lymphatic trunks in the portal fissure, but his best results were obtained by the employment of a method suggested by Fleischl, viz., the puncture by the fine nozzle of the injecting syringe of the wall of one of the hepatic veins. By this procedure the injection mass is forced into large lymphatic vessels which lie deeply in the wall of the vein, and it can readily pass from them through the liver to issue by the efferent lymphatics in the portal fissure. Budge was the first to describe the lymphatics of the hepatic veins, and to show that they are very numerous in this situation. He stated that the trunks are large, and have no valves; in the walls of the large veins there may be from 60 to 70 trunks, in the medium sized veins 15 to 20, and from 3 to 5 in the small, and they are lined with endothelial cells. Budge found that the injection passed through the walls of the lobules of the liver in spaces lying between the walls of the blood-vessels and the liver cells, and opened into the large vessels accompanying the branches of the portal vein. The spaces, he argued, must be lymphatics, because they afford the only means of communication between the hepatic and portal lymphatic trunks.

In 1876 Kupffer (37) described in the liver the star-shaped cells which have since borne his name. He believed them to be connective-tissue cells lying outside the blood-vessels of the lobules, and related in all probability to the origin of the lymphatics.

Heidenhain (22), in 1881, spoke of Kupffer's cells as taking a probable part in the formation of perivascular lymphatics.

Disse (15), in 1890, published the results of a number of experiments in which the liver lymphatics were injected by Fleischl's method. Disse removed the liver immediately after death, opened the hepatic veins and introduced the nozzle of the syringe through the wall of one of the veins from the inside. Watery solutions of Prussian blue, and in one case a 0·75-per-cent. solution of silver nitrate were injected, and in many of the experiments the blood-vessels were subsequently filled with carmine gelatine from the portal vein. After the injection was completed the liver was fixed and hardened in alcohol. Disse reviewed the work of previous observers, and raised the objection to most of it that, although spaces had been injected, it had not been shown that they had definite walls bounding them. He held that it was neccessary to prove that the spaces have demonstrable walls which can be shown without filling them with injection mass. Disse's results correspond very closely with those obtained by Budge. He found large lymphatic trunks in the walls of the hepatic veins, and agreed with Budge that they are lined by endothelial cells. The injection material introduced into the wall of the vein quickly spreads under a low pressure, and emerges by trunks in the portal fissure. Only a small portion of the liver in the neighbourhood of the puncture is injected, and in this situation the injection mass penetrates into the lobules and follows the blood-vessels, filling clefts between them and the liver cells, and uniting outside the lobules with the lymphatics of the portal spaces. Disse could find no endothelial cells lining these clefts, but nevertheless believed them to be lymphatics. Disse, however, found that there is another means of communication between the hepatic and portal lymphatics by large trunks running between the lobules in connective-tissue septa which unite the adventitia of the hepatic veins to the connective tissue of the portal spaces.

Disse also examined sections and teased preparations of healthy livers, and came to the conclusion that the clefts round the capillaries of the lobules are the lymph radicles of the liver parenchyma, and that their walls consist of structureless ground substance and fine fibrils of an equal thickness forming a membrane which is paved at intervals with the star-shaped cells that surround the capillaries. The liver cells, he stated, are contiguous, on the one side with bile capillaries, on the other with lymphatic spaces ; and the lymph flow from the lobules can go in two directions, by the portal vein system which emerges from the portal fissure, and by the hepatic system which runs through the diaphragm to lymphatic glands in the posterior mediastinum.

In 1896 Teichmann (60) made an additional contribution to the literature of the subject. He described the lymphatics of the liver as forming networks which surround the branches of the portal vein, but nowhere enter the lobules. He failed to find lymphatics in the walls of the central veins of the lobules and in the walls of the hepatic veins.

In 1898 Reinke (51) described in the liver lobules connective-tissue cells other than Kupffer's cells. He also stated that he had seen the lymph spaces portrayed by Disse lying between the capillaries and the liver cells, and that the connective-tissue cells form sheaths for them ; perhaps a lymphatic endothelium.

In the same year Kupffer (38) took up a new position regarding the star-shaped cells he had described in 1876. He now considered them to be endothelial cells of a peculiar nature which belong to the walls of the blood-vessels, and not connective-tissue cells lying outside the blood-vessels.

Browicz (11), at the same time and independently of Kupffer, arrived at a similar conclusion, but went further in regarding these cells as having an intravascular situation. He also stated that the existence of perivascular lymphatics inside the liver lobules is doubtful. In subsequent papers Browicz emphasised the fact that the connection of the liver cells with the blood capillaries is much more intimate than has been generally supposed, and insisted that in all probability the perivascular lymphatics described by MacGillavry and others within the liver lobules do not exist.

Holmgren (28), in 1902, found lymph channels in the liver cells of the hedgehog, and considered that they are in direct communication with lymph spaces lying between the liver cells and the blood-vessels.

In 1902 Schäfer (55) showed that the liver cells can be injected from the portal vein, and that there is a direct communication by means of fine channels between the lobular capillaries and the interior of the liver cells. He could find no trace of perivascular lymphatics within the lobules, but described the injection as having passed in a diluted form into the lymphatics accompanying the branches of both the portal and hepatic veins.

Several authors have argued the presence of perivascular lymphatics in the lobules from the results of work by Fleischl (19), Kunkel, Kufferath (36), and Vaughan Harley (21). These observers showed that ligature of the bile duct in living animals is followed by escape of bile, not directly into the blood, but into the lymphatics of the liver. Ebner* and Oppel (1900) considered that the bile after ligature of the common bile duct finds its way into lymphatics inside the lobules, and is prevented by them from entering the blood-vessels directly. Such a view assumes that the leakage of bile takes place within the lobules either from the liver cells direct or from the bile capillaries, and that the bile is under these circumstances taken up by intralobular lymphatics and passes from them into the lymphatics accompanying the portal vein branches.

Heidenhain (22), in 1881, showed that in jaundice experimentally produced in animals the place where the bile is absorbed by the lymphatics does not coincide with the place where the bile is formed. He found that a pressure of 11 to 15 mm. Hg in the common bile duct causes bile to appear in the lymphatics of the liver, that secretion of bile continues although this absorption is taking place, and that after the obstruction is removed the bile which escapes from the bile duct is not more concentrated than it was before, showing that equal proportions of the solid and watery constituents of bile were passing into the lymphatics. He also showed that in experimental jaundice the lobules of the liver are not stained with bile unless the biliary obstruction is one of long-standing duration and, in such cases, he argued that the bile finds its way from the lymphatics into the lobules by perivascular lymphatics, and so into the liver cells. Heidenhain believed in the existence of intralobular lymphatics, but considered that they become filled with bile secondarily, and then only in cases of long-standing biliary obstruction where pathological changes have taken place leading to obstruction of the larger lymphatics. In experimental jaundice he stated that the bile breaks through the walls of the interlobular bile ducts into lymphatics around the vessels, and never enters the interior of the lobules.

Nauwerck (46), in 1897, criticised Vaughan Harley's results, believing that they did not prove that bile always finds its way directly into the lymphatics when the bile duct is obstructed. Nauwerck stated that in cases of obstructive jaundice in man the *post-mortem* examination of the liver gives no foundation for the view that the bile passes directly into the lymphatics, but that all appearances support the view that the bile passes through the liver cells by a network of fine channels and escapes directly into the lobular blood-vessels.

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* Kölliker's 'Gewebelehre,' 1899.

Nearly all the authors above quoted agree in describing the existence of lymphatic spaces encircling the intra-lobular blood capillaries. Hering is the strong opponent of this view, and he was supported to some extent by Kölliker. Most of its advocates have relied on the results of injection of the lymphatics with watery solutions of Prussian blue, asphalt dissolved in chloroform, or alcannin in turpentine oil. These fluids have been described as making their way along clefts or spaces between blood-vessels and liver cells throughout the lobule. The readiness with which injection material passes from ruptured lymphatics into the blood-vessels has been generally admitted, but its full extent cannot be appreciated when the blood-vessels are injected subsequently with material of another colour, and this method of procedure has been adopted by nearly all workers on the subject. Biesiadecki acknowledged that in the human livers he investigated the injection material found its way into blood-vessels and clefts equally. Had he injected the blood-vessels with another material this important fact would have been disguised, and the typical appearance described by MacGillavry and others would have been obtained. Budge recognised this difficulty, and stated that the first injection employed has a tendency to adhere to the vessel walls, but he believed that a double injection of a blood-vessel could be distinguished from the separate injection of blood-vessel and lymphatic by the sharp definition which exists in the latter case between the two. He described the outlines of the lymphatic injections as smooth and sharp. Budge employed asphalt in chloroform to inject the lymphatics. We made use of a similar solution to inject the blood-vessels, but found it most unsatisfactory. The chloroform evaporates or diffuses away and leaves the asphalt adhering to the vessel walls. It also alters the character of the liver cells and causes shrinkage of the tissues with which it comes into contact, and another great disadvantage in its use is its lack of colour when seen in thin sections. In dealing with the question whether fine clefts exist between blood-vessels and liver cells the injection material used must be such as can be detected in the thinnest of sections, and asphalt does not admit of this. The forcing of chloroform into a tissue is, besides, not unlikely to produce artificial clefts by the shrinkage which it induces, and these clefts will be accentuated by subsequent fixation and hardening.

Watery solutions of Prussian blue have been extensively used; they have the fatal property of diffusibility. Carter (14) urged this objection to the use of Prussian blue. It is, moreover, readily decolourised by the tissues, and although the colour can be restored, it is, as Carter pointed out, both soluble and diffusible in its colourless condition.

Kisselew and Reinke alone of all who have worked on the subject have described endothelial cells lining the clefts between intralobular blood-vessels and liver cells. No one else has been able to find them. Hering denied the existence of natural clefts in this situation in the rabbit's liver, but allowed that they might be produced artificially.

Methods Used in the Present Inquiry and Results Obtained.

The observations of the brothers Fraser, Nauwerck, Browicz, and Schäfer, and the results of our own experiments on injection of the liver cells from the blood-vessels, are against the probability of there being perivascular lymphatics in the lobules of the liver, but are not conclusive in themselves. We have injected the lymphatics of the liver to ascertain if we could get the injection mass to run into the clefts described by MacGillavry.

The animals experimented on were dogs and cats. The injection mass used was carmine gelatine prepared as already described for injection of the blood-vessels. Carmine gelatine is pre-eminently suitable for this purpose on account of its lack of diffusibility, the readiness with which it is solidified and fixed without damage to the surrounding tissues, and the ease with which the smallest amount can be detected. The main lymphatic trunks coming from the portal fissure were clamped immediately after death; they soon become distended with lymph. One of the larger trunks close to the liver was selected, and a thread passed round it by means of a needle, care being taken not to injure any neighbouring trunks or the portal vein. It was then opened, and a fine cannula, connected to the injection bottle and filled with carmine gelatine, was inserted and tied in position. The liver was disturbed as little as possible. The whole animal being immersed in a bath of water at the temperature of the body, the pressure was gradually raised until the carmine gelatine began to distend the lymphatic vessel. The pressure was maintained for from one to three hours. In most cases the inferior vena cava was opened above the diaphragm to prevent any rise of pressure in the blood-vessels. When the experiment was completed the liver was removed from the body, the clamp on the efferent lymphatic vessels being still attached; the tube which conducted the injection mass to the lymphatics was ligatured close to the cannula. These precautions were adopted to prevent any escape of injection fluid from the lymphatics. The liver was at once placed in cooled 10-per-cent. formalin, and as soon as the gelatine had set was cut into small pieces and replaced in the formalin. Sections were subsequently cut in paraffin and stained with dilute hæmatoxylin.

The injection can be made to pass beyond the valves without any rupture of the vessel wall taking place; it usually spreads first to the neighbouring trunks and then enters the liver substance. We find no evidence of superficial lymphatics on the surface of the liver in the dog or cat, and in this respect agree with MacGillavry, who could not find them in the dog or rabbit. The large trunk which MacGillavry found on the surface of the gall bladder is readily filled if the injection is made in the vicinity of the gall bladder. This trunk receives its radicles from the connective tissue around the gall bladder and pursues a tortuous course along the cystic duct, to end in a lymphatic gland near the portal vein. Another large trunk is often filled which runs from the connective tissue of the portal fissure along the round ligament into the suspensory ligament of the liver; it appears to terminate by joining the lymphatics of the diaphragm, but we have not been able to trace it further. This lymphatic trunk is evidently similar to the one described by Teichmann and others; it arises, however, not from surface lymphatics, but from the connective tissue of Glisson's capsule, and is closely related to the efferent lymphatics which accompany the portal vein. It affords for the lymph of the liver an additional outlet by means of the lymphatics of the diaphragm.

One of the most remarkable features of an injection of the liver lymphatics is the absence of any sign of the injection mass on the surface of the liver; vessels appear close to the fundus and at the sides of the gall bladder and join its central trunk, but they are distinctly confined to the connective tissue separating the gall bladder from the liver, and no vessels join them from the surface of the organ. After a prolonged injection we have found the wall of the inferior vena cava above the diaphragm coloured to a certain extent by the presence of carmine gelatine in it, but the flow along the wall of the inferior vena cava is very slow and does not spread far.

Whenever the liver has shown a distinct appearance of injection on its surface, it has, in our experience, been due to rupture into and injection of the blood-vessels. In such cases the injection mass may or may not have entered in sufficient quantity to reach the opened inferior vena cava.

On cutting up a liver, the lymphatics of which have been injected in the above described way, the injection mass is seen at many points in its substance close to or surrounding the blood-vessels in the portal spaces. In the neighbourhood of the portal fissure there is often a considerable amount of extravasation of the carmine gelatine, principally in the connective tissue accompanying the blood-vessels and bile ducts, but also in some of the lobules. Injection mass is also seen in the walls of the hepatic veins.

Microscopical examination of sections lightly stained with hæmatoxylin shows that the carmine gelatine is in the connective tissue of the liver, and that large injected trunks accompany the branches of the hepatic artery, portal vein, and bile ducts. In the large portal spaces there is frequently extravasation throughout the connective tissue; the large lymphatic trunks can be distinguished by their being filled with carmine gelatine, but the surrounding connective tissue is lightly stained owing to the presence in it of diluted injection mass.* The carmine gelatine is frequently seen extending into some of the lobules even to the central vein, and presents an appearance very like that described by MacGillavry. In other places no trace can be seen of injection passing into the lobules, although extravasation has occurred throughout the connective tissue bordering them; a sharp line of demarcation exists between injected connective tissue and non-injected periphery of lobule.

In the smaller portal spaces injected lymphatics are sharply defined and extravasation is uncommon. Lymphatics are often seen running parallel with and close to branches of the hepatic artery; they also surround the bile ducts and portal vein. Even in the smallest portal spaces lymphatics are found (fig. 14). The walls of the hepatic veins contain a large plexus of lymph vessels lying principally in the adventitia close to the liver substance, but there may be several layers with anastomosing branches (fig. 16). The mode of connection between the portal lymphatics and those of the hepatic veins is easily seen. Branches of the hepatic artery supply the walls of the hepatic veins, and lymphatics accompany these branches. Further, large portal spaces containing hepatic artery, portal vein, and bile ducts are found in close connection with, indeed joined on to, the walls of the hepatic veins (fig. 13). There is, in fact, an intimate relationship between Glisson's capsule and the adventitia of the hepatic veins, and the large lymphatics of the two systems are directly continuous in these situations. The place of entry of the branches of the portal vein into the liver is, moreover, very close to the large hepatic veins, and the lymphatics of the latter join the large lymphatics which accompany the branches of the portal vein and which leave the liver by the portal fissure. Disse, alone of all who have previously worked at the subject, described this connection between the two systems. Budge did not notice it, and was led to believe

* Is it possible that on dilution with alkaline lymph some of the carmine becomes diffusible?

in intralobular lymph clefts, largely because he could not find any other connection between portal and hepatic lymphatics. Disse described large lymphatic trunks lined by endothelial cells which run in connective-tissue septa between the adventitia of the hepatic veins and the connective tissue surrounding the branches of the portal vein, but he did not emphasise the fact that the lymphatics also accompany the branches of the hepatic artery.

The plexus of lymphatic vessels in the adventitia of the hepatic veins follows them to their small branches, and lymphatics are present wherever there is an appreciable amount of connective tissue in the wall of the vein. In transverse section of the smaller hepatic veins several lymph vessels (fig. 15) are seen in their walls. The number of these channels varies, as Budge stated, according to the size of the vein they surround; the larger the vein the greater the number of lymphatics in its wall.

We have employed Fleischl's method of injection in several experiments, using the same apparatus as before, but removing the liver from the body and introducing the point of a fine cannula into the adventitia of one of the hepatic veins. The injection mass causes a local distension of the wall of the vein, and some extravasation takes place, but the injection gradually finds its way into the lymphatics and appears at the portal fissure. The extent of liver injected by this method is very small. Disse, in his injections, made several punctures in different places, and noted the small areas of liver injected. This is readily explained, and is what one would expect. The lymphatics of the large branches of the hepatic veins have frequent and large communications with the lymphatics of the branches of the portal vein, and the injection mass has a ready means of escape and soon appears in the large efferent trunks at the portal fissure; it spreads very little and simply follows the large lymph channels. The natural flow of lymph from the hepatic veins in all probability takes the same course. Very little spread of injection mass is seen towards the inferior vena cava, and our observations point to the probability that most of the lymph of the liver in the dog and cat emerges at the portal fissure.

It has been mentioned already (p. 476) that after injection of the portal lymphatics some of the lobules, especially those in the neighbourhood of the portal fissure, contain injection mass, and present appearances similar to those figured by MacGillavry. On careful examination of well-fixed specimens in thin sections it is apparent that the injection is undoubtedly inside the blood-vessels, not only in the intralobular vessels, but in neighbouring interlobular branches of the portal vein as well. Sometimes the periphery only of a lobule shows the injection, extending inwards a little way from the portal space; in such cases the interlobular portal vein nearly always contains some carmine gelatine mixed with the blood corpuscles. Wherever comparatively little injection mass has entered the intralobular blood-vessels, it shows a tendency to adhere to the walls; this gives rise to the appearance of a thin layer of injection close to the liver cells. The injection is frequently found in Kupffer's cells on either side of their nuclei, but Kupffer's cells show exactly the same appearance in imperfect injections of the blood-vessels, and it is not uncommon to find in the normal liver red blood corpuscles lying in the same situation. In many lobules the intralobular vessels are comparatively well filled; in these cases many of the liver cells, both in the dog and the cat, contain the carmine gelatine, but in all cases where the liver cells contain the injection there is no doubt of its presence in the interior of the neighbouring blood-vessels. On the other hand, there are places where the portal spaces are filled with carmine gelatine and not a trace of any has spread into the adjacent lobules. The same is seen after injection from the wall of the hepatic vein; there is often well-marked extravasation into the connective tissue of the adventitia, but not a trace of injection passing into the surrounding liver tissue. The usual site of rupture into the blood-vessels appears to be at the borders of the lobules where the interlobular veins break up to enter between the columns of liver cells. The usual site of extravasation of bile after ligature of the common bile duct is, according to Heidenhain, just outside the lobules, and this appears to be the place where both blood-vessels and bile ducts are weakest; their walls lack the support they receive from the liver cells in the lobules, and have not attained the strength they afterwards possess when united to form the interlobular veins and bile ducts.

In several animals in which we injected the bile ducts, rupture obviously took place in this situation. The injection mass passed into both blood-vessels and lymphatics of the interlobular connective tissue, and did not fill the bile capillaries at all. The passage of bile into the lymphatics of the liver after ligature of the common bile duct in living animals is not an argument in favour of the presence of intralobular lymphatics. It is more reasonable to suppose that rupture takes place into the connective tissue just outside the lobules, and that the bile under a comparatively low pressure finds its way more readily into the lymphatics than into the blood-vessels. When the bile ducts are injected at a pressure higher than can be reached in simple obstruction of the common duct (11 to 15 mm. Hg according to Heidenhain), rupture takes place into the blood-vessels.

We do not believe that the injection appearance in the lobules denotes the presence of lymphatic clefts between the blood-vessels and liver cells;

the lymphatics of the liver are, in our opinion, confined to the obvious connective tissue which accompanies the branches of the portal and hepatic veins; the appearances described by MacGillavry are produced by artificial rupture into the blood spaces.

The parenchyma of the normal liver contains little or no connective tissue; fine fibrils, the "Gitterfasern" of Kupffer, have been shown to exist by many observers, but they are extremely fine and closely applied to the liver cells. We can find no evidence of the existence of any perivascular connective-tissue cells in the lobules. The "Gitterfasern" are more evident in man and the dog than in the cat and rabbit. In the two latter animals it is often impossible to observe any indication even of an endothelial outline between the blood and the liver cells, except where Kupffer's cells occur. Minot has shown that the connective tissue appears late in the development of the liver, and that it is accompanied by the appearance of true capillaries which grow from the hepatic artery. To us it seems probable that the lymphatics of the liver have a similar distribution, and are specially related to the hepatic artery and its branches. As has already been stated, Minot has shown that the blood spaces of the liver are originally sinusoids, and one of the characters of a sinusoid is the non-occurrence of connective tissue between its wall and the adjacent parenchyma. Under these circumstances the wall of the blood space is closely applied to the (liver) cells and there is no natural cleft between them, nor is there, as in other secreting glands, an intervening lymph space. And if, as appears certainly to be the case in some animals, the endothelial wall of the blood space is itself deficient or is only represented by the isolated cells described by Kupffer, the liver cells must be in direct communication with the blood and the blood plasma, and even under some circumstances blood corpuscles may pass directly into the cells.

It is known that a large amount of concentrated lymph comes from the liver, and Rutimeyer (54) found solid particles of cinnabar in the efferent lymphatics of the liver 35 minutes after a suspension of cinnabar in salt solution had been injected into the external jugular vein of a dog; a very intimate relationship between blood-vessels and lymphatics must therefore also exist in the liver.

To explain this relationship, and the ready passage of injection material into the liver cells, it is necessary to consider the structure of the walls of the hepatic capillaries in some detail.

The Structure of the Intralobular Blood-vessels. (*Historical.*)

His (27), in 1860, described the capillaries of the liver and remarked that they differ from the capillaries of other parts of the body in that their walls present an indistinct contour which is due to their having closely applied to them a thin layer of connective-tissue fibres. The fibres are extremely fine and occasionally stretch from vessel to vessel; the capillary wall consists of a layer of endothelial cells separated from the liver cells by the fine fibres only.

Wagner (61), in the same year, described in the liver a delicate membrane everywhere surrounding the hepatic cylinders. It is different from the capillary membrane, but is in close relationship with it, so that only one delicate membrane separates the blood from the liver cells. The membrane surrounding the liver cells he stated to be perfectly clear and homogeneous, and of the greatest delicacy and transparency when normal. It has also a further and peculiar property in possessing nuclei, and small cell-like structures in its wall. The nuclei are constantly present, but differ in number and arrangement; they are for the most part round and seldom elongated. Other cells of an irregular or pointed shape occur, and belong to the same membrane. A visible cement substance between capillary wall and membrane of liver cells does not exist, but the two membranes are closely applied to one another.

Wagner pointed out that the liver differs from nearly all other organs in the absence of connective tissue within its lobules. He laid emphasis on the fact that the connective tissue of the liver never penetrates into the lobules under normal conditions, and that any thickening of the membrane surrounding the intralobular capillaries is of a pathological nature.

In 1864, MacGillavry (42) described a space between capillary wall and liver cell which he regarded as one of the sources of the lymphatics of the liver. He found that the space is lined by connective-tissue fibres on the side of the blood capillary and by liver cells and the walls of bile capillaries on the other side. MacGillavry, therefore, differed from previous observers in regarding the walls of the blood capillaries as having a less intimate relationship to the liver cells, and being separated from them by perivascular lymphatics.

About the same time Carter (13) described in the capillary walls of the liver nuclei which are filled with injection when carmine gelatine is injected into the blood-vessels. He came to the conclusion that the nuclei instead of being simply embedded in the walls of the capillaries are nucleated tubular swellings which connect the blood-vessels with a "diaplasmatic system" of vessels. Carter described plasmatic channels between the liver cells and called them a "diaplasmatic system of vessels" or "intercapillary plexus." He considered that blood plasma alone passes into them and that the nucleated sheath of the blood-vessels allows the ready permeation of plasma, but not of red blood corpuscles. He also believed that nucleated fusiform cells in the capillary walls give rise to the lymphatics of the liver.

Carter's paper does not appear to have been seen by many of the subsequent workers, and is seldom mentioned in the literature.

Hering (25), in 1866, criticised MacGillavry's work and was firmly convinced that in the rabbit's liver no space exists between the capillary walls and liver cells. Hering described the neighbouring liver cells as being connected together by a "Scheidewand," which, when seen in profile, has the appearance of a single dark line or a fine double contour with clear space between. The last, he said, is not to be mistaken, as is done by MacGillavry, for the contour of a natural canal; it is due to the "Scheidewand" not being cut in profile. Whether the wall is composed of cement substance between the closely lying cell membranes or of a homogeneous substance is unsettled. In an alcohol-hardened preparation Hering said two cells always separate so that the protoplasm of one cell at least is torn away from the common "Scheidewand." He could find no trace of any membrana propria in the rabbit's liver and believed that the capillary wall is closely adherent to the liver cells. The perivascular lymphatics described by MacGillavry, Hering believed to be non-existent in the normal liver. He also stated that MacGillavry was wrong in describing bile capillaries between blood-vessels and liver cells.

Kölliker (35) recognised connective-tissue cells in the lobules, and a fine ground substance which accompanies the capillaries, but which is very scanty in amount, and only revealed by special stains.

Henle (24) denied the presence of intralobular connective-tissue cells, but found fine threads between the capillaries and liver cells.

In 1869, Ponfick (49) showed that after fine particles of cinnabar have been injected intravenously in rabbits, guinea-pigs and dogs some hours before death, a *post-mortem* examination of the liver shows that many of the granules of cinnabar lie in round or oval amœboid cells which may be mistaken for liver cells. Ponfick described cinnabar-holding cells in the interlobular connective tissue, and in the fine connective-tissue sheath of the intralobular capillaries in what he considered an extravascular situation.

Platen (48) also described cells in the liver lobules which take up fat from the blood, and may be filled with fat droplets.

In 1876, Kupffer (37) described "Sternzellen," or star-shaped cells, in the liver; these cells are now known as Kupffer's cells. Kupffer stained sections of liver with gold chloride, and found that certain cells take up the stain, and show as deep black bodies on a red field. He described them as pointed protoplasmic bodies, varying in size, but always much smaller than the liver cells, and containing one or more nuclei. They are regularly distributed throughout the lobules, and occur at intervals along the vessels, never in groups. Their position is constant in being always in contact with the capillary wall; but the form of contact varies. Some cells enclose the capillary with their processes, others have one end only touching the vessel while the main body lies on the nearest liver cell; their branches often pass in between the liver cells, and may even reach the bile capillaries.

Kupffer found "Sternzellen" in the liver of the rat, mouse, rabbit, pig, dog, and man, and believed them to be connective-tissue cells lying outside the blood capillaries. He identified them with the cells described by Wagner and Kölliker, and with some but not all of the cinnabar-holding cells of Ponfick, and thought they might prove to be related to the origin of lymphatics. Kupffer also described an intralobular scaffolding of fine non-nucleated, sharply-cut fibres, which, running from the sheath of the central vein, break up into fibres of extraordinary fineness and support the portal vein capillaries throughout the lobule. To this intralobular network of fibres he applied the name "Gitterfasern." Many subsequent observers have corroborated Kupffer's views, but there has been some difference of opinion regarding the character of the cells he described. Ehrlich (18) looked on them as belonging to his group of plasma-cells. Heidenhain (22), on the other hand, regarded them as special connective-tissue cells related, in all probability, to the perivascular lymphatic spaces described by MacGillavry Ribbert (52) laid stress on the property they have of taking up from the blood not only solid particles introduced into it, but also of depositing in their protoplasm granules from materials which enter them in a soluble form.

Rothe (53) found Kupffer's cells in the cat, guinea-pig, sheep, and sparrow; Asch (2) found that they rapidly take up particles of cinnabar and carmine when these are injected into the blood. In the liver of the frog he states that the pigment cells of the liver have the same function. Löwit (41) concluded that Kupffer's cells take up red blood corpuscles, and transfer the hæmoglobin to the liver cells.

Biondi (6) and Lindemann (40) state that Kupffer's cells take up iron-containing pigment in pernicious anæmia and after the intravenous injection of substances like toluylendiamine. Arnstein (1), in 1874, described pigment-holding cells in connective tissue between capillaries and liver cells in cases of melanosis. The phagocytic property of Kupffer's cells has been amply proved by further researches of Kupffer himself, by Rutimeyer (54), Siebel (57), and quite recently by Heinz (23). Kupffer at first, and nearly all subsequent observers, regarded the "Sternzellen" as extravascular cells lying between capillary walls and the liver cells. Disse looked upon them as helping with connective-tissue fibrils to form the walls of perivascular lymphatics.

In 1898, Kupffer (38) returned to the subject, and stated that he had been mistaken in assigning to the "Sternzellen" an extravascular position, he now believes that they are an integral part of the blood capillary wall. Kupffer found that 12 hours after the injection of defibrinated (rabbit's) blood into the vein of a rabbit, red blood corpuscles are to be seen lying inside the endothelial cells of the capillaries of the portal vein. In older animals, he states, it is not rare to find the capillary walls thickened, and the injection of perivascular spaces from the lymphatics may be due to the injection mass lifting off an adventitial layer from the capillary tube. As a result of his later observations Kupffer concluded that, in the capillary walls of the liver, there are two forms of nuclei, one spherical or ellipsoidal and the other quite flat; the large nuclei belong to cells which are rich in protoplasm, the small flat nuclei to cells which have relatively little protoplasm. The endothelium of the capillary wall has the appearance of a syncytium, and it is impossible to demonstrate limits between individual cells. The capillary wall appears to be made up of a continuous thin lamella in which the protoplasm is arranged as a network of threads with nuclei-holding nodes; larger accumulations of protoplasm surround the large nuclei, and there is less protoplasm round the flat nuclei. The "Sternzellen" belong to the endothelium of the portal vein, and in gold preparations are shown up by the arrangement of protoplasm round the endothelial nuclei. This protoplasm possesses the power of phagocytosis, and foreign bodies and red blood corpuscles are taken up by it from the blood and broken into fine particles.

About the same time that Kupffer's later results appeared, Browicz (11) described in the human liver and in the liver of the dog long and voluminous cells which lie close to the capillary wall and project into its lumen. He found that these cells are phagocytic, and frequently contain red and white blood corpuscles and granules of pigment, and he identified them with cells containing blood corpuscles found by Silbermann in the liver-blood of children suffering from jaundice, and with phagocytic cells also occurring free in the liver-capillaries of ducks and geese, and described by Minkowsky and Naunyn. Browicz states that the cells do not form a continuous layer on the wall of the capillary, and that the latter is sometimes seen lying between them and the adjacent liver cells; in some pathological conditions they even give rise to emboli in the blood-vessels.

In a later paper Browicz (8) identified the cells he had described with those shown by Kupffer, but insists that they do not form a syncytium, but are well defined cells which occur inside the blood-vessels. He also considers that the blood has a very close relationship to the interior of the liver cells, and argues against the presence of perivascular lymph spaces in the liver lobules.

Reinke (51), in the same year, described a fine membrane which surrounds every liver cell like a capsule, and is composed of connective-tissue cells, some of which resemble Kupffer's cells, while others are more like the tendon cells seen in the tail of the mouse. He also believes in the existence of the perivascular lymph sheath described by Disse, and thinks that the connective-tissue cells take part in the formation of its wall, and are probably endothelial cells lining it.

Little has been said about the lining of the capillary walls by most of the authors who have described Kupffer's cells as extravascular connective-tissue cells.

Ranvier (50), in 1885, described the capillary walls as composed of a granular and very thin sheet, in which nuclei occur at intervals; the latter are flattened, with long axis parallel to the long axis of the vessel, but with a pronounced relief on their internal surfaces. Ranvier could find no cell margins, and explains the absence of impregnation lines after using silver nitrate in the liver by assuming that endothelial cells are lacking, and that the cells which form the walls of the capillaries are embryonic in character and form a syncytium of protoplasm enclosed by two homogeneous sheets containing nuclei at intervals.

Ranvier's views and the later ones of Kupffer are very similar: both believed that the walls of the capillaries are formed by a syncytium in which there are nuclei but no separate cells. Kupffer showed that the amount of protoplasm round the nuclei varies in amount, and that it has active phagocytic properties. Other observers regarded Kupffer's cells as extravascular connective-tissue cells, as Kupffer himself did at first. Browicz, on the other hand, assigned to them an intravascular position.

Berkley (5), by the employment of a modification of Golgi's method, found two kinds of perivascular cells in the liver of the rabbit, and Dogiel (16), by a similar method, described cells investing the capillaries with their branches.

Many observers, from His onwards, have described fine connective-tissue fibres between the capillary walls and liver cells in the lobules. They are demonstrated for the most part by special stains only, and received from Kupffer the name of "Gitterfasern," a term which Oppel (47) revived in 1891. Oppel employed a method of staining by silver nitrate, and described radial fibres passing from interlobular connective tissue to central vein, and investing fibres which are finer in texture and surround the blood and lymph spaces. The radial fibres are the thicker and coarser of the two sets.

Mall (43) described similar appearances in the lobules after removal of the liver cells by artificial pancreatic digestion. The nature of the fibres is uncertain. Kupffer states that they have the appearance of elastic fibres, but cannot certainly be classified as such, as they are not stained with orcein. Mall believed them to be elastic. Ebner (17) showed that in preparations stained with orcein, fibres in the interlobular connective tissue, which are undoubtedly elastic, take on a deep stain, while the "Gitterfasern" are unaffected. He believes them to be composed of collagen, and classifies them as fine white connective-tissue fibrils.

In 1900 Minot (45) described a hitherto unrecognised form of blood circulation without capillaries in the organs of vertebrata. Among other organs the liver is one in which, according to Minot, the blood-vessels of the lobules are not true capillaries, but blood spaces or "sinusoids." He states that a sinusoid differs from a capillary in that its wall consists of an endothelial or endotheloid layer without any strengthening addition of adventitia or media. It is of relatively large size; its epithelium is closely fitted against the cells of the organ in which the sinusoid is developed, and it has numerous wide and frequent communications with the neighbouring sinusoids of the organ, while a capillary follows its own shape and is chiefly or wholly embedded in connective tissue. A sinusoid, on the other hand, has little or no connective tissue between it and the adjacent parenchyma, and in those cases where this occurs it is a secondary or late acquisition, and the amount remains usually, perhaps always, very small. The development of capillaries and sinusoids differs. Minot states that a capillary arises from pre-existing vessels or from vaso-formative cells, and is an addition by new histogenesis to vessels previously differentiated, while a sinusoid, on the contrary, is not the product of a new histogenesis, but results from ingrowth of the endothelial wall of a pre-existing blood-vessel. In the development of the liver the hepatic cylinders grow into and subdivide the portal vein, the endothelial cells retaining their embryonic character, and being closely applied to the ingrowing columns of liver cells. The blood-vessels or sinusoids between the columns have irregular shapes and numerous connections with one another, and are typically many times wider than capillaries. Minot states that in the liver the sinusoids come to resemble capillaries by secondary change, and may be called "capilliform sinusoids." The fine fibres of connective tissue he believes to be a secondary development, but one that does not change the essential intimate relation of the wall of the sinusoid to the adjacent parenchyma.

Minot further believes that the supposed endothelial cells lining the liver sinusoids do not form a true endothelium, but a layer of widely separated mesenchymal cells, and he states that the physiological processes that take place between the blood of a sinusoid and its adjacent parenchyma occur under conditions very different from those where the blood flows in true capillaries.

Ebner, in the last edition of Kölliker's "Gewebelehre" (Bd. 3, S. 664, 1902), admits that the embryonic liver contains wide capillaries, but does not think that the fully-formed liver agrees with Minot's description, and regards the employment of the term "sinusoid" as superfluous.

In 1904 Lewis (39) emphasised the importance of Minot's discovery. Lewis re-affirmed the particulars regarding the development of the liver given by Minot, and states that the liver contains at first the smallest possible amount of connective tissue, but that later an increase of connective tissue along the inferior vena cava transforms certain sinusoids into veins, while another and more extensive growth along the bile ducts and portal veins takes place, but never spreads into the lobules. Sinusoids persist between the hepatic columns throughout life, and the so-called central veins of the lobules are large vessels which replace several smaller ones, and in structure remain sinusoids rather than veins.

All who have worked at the subject are agreed that the blood-vessels inside the liver lobules present special features. There is a unanimity of opinion that very little connective tissue exists between their walls and the adjacent liver cells. Most recent observers agree that there is a scaffolding of fine connective-tissue fibres which can only be seen after the employment of special stains. The connective-tissue cells described by earlier authors have been identified as Kupffer's cells, and shown to possess special phagocytic properties; they have been proved, moreover, by Kupffer himself, by Mayer, Browicz, and Minot to enter into the formation of the walls of the blood-vessels. Ranvier and Kupffer believed that the walls are composed of a syncytium with nuclei at intervals and accumulations of protoplasm around them, varying in amount in different places. Browicz described cells actually projecting into the blood-vessels.

Hering could find no space between the cells lining the blood-vessels and the liver parenchyma. Kupffer latterly, Browicz, Schäfer, and Minot have insisted on the same thing. On the other hand, MacGillavry and many subsequent observers claim to have injected perivascular lymph spaces, and Kisselew and Reinke describe lymph spaces lined by endothelial cells. Hering believes such injections to be artefacts, and Kupffer states that they might be due to the injection lifting off an adventitial layer from the capillary tube.

Results of our own Observations.

We have been unable to find any perivascular lymph spaces in the lobules, but have seen appearances which might be mistaken for such; in every such case we have been able to satisfy ourselves that the injection is inside the wall and not between it and the liver cells.

We agree with Minot that the blood-vessels have more the character he assigns to sinusoids than that of capillaries. In injection preparations the distended blood-vessels follow the outlines of the liver cells, and at places penetrate for some distance between them. This appearance was described by Carter, and by J. W. and E. H. Fraser. In the liver of the bird it is extremely well marked (fig. 9), but it is present in all livers we have examined. In injected preparations of the livers of fœtal rabbits the blood-vessels appear as large sinuses closely investing the liver cells and passing between them. Wherever the injection penetrates between the cells it shows a broad connection with the injection mass in the lumen of the blood-sinus and gradually tapers to a fine point between two liver cells (figs. 4 to 9). There is no evidence of any wall lying between the intercellular injection and the lumen of the blood-vessel.

The cells which occur at intervals along the blood-vessels may be divided into two classes, which agree with those described by Kupffer. Small nuclei with very little protoplasm are found closely applied to the liver cells; they have the general appearance of endothelial cells, and their long axes are parallel to the direction of the vessel which they line. One of these cells is shown in fig. 3. They do not appear to form a continuous sheet of endothelium, and long intervals frequently occur between them.

The other class is composed of much larger cells with comparatively large amounts of protoplasm. They vary in shape, and in section may appear as long, pointed cells or star-shaped with several processes. Each cell contains a nucleus which is usually elongated, but varies in size and shape in different cells. Occasionally a large nucleus projects into the lumen of the vessel and lies with its base closely applied to the wall of a liver cell (fig. 3). The protoplasm round the nucleus is sometimes difficult to see, in which case it looks as if nucleus alone were present, but in other cases the protoplasm is large in amount and granular. We take it that these are Kupffer's cells, but they frequently have the appearance described by Browicz, of projecting into the lumen of the vessel, and in thin sections may even seem to be lying free. Fig. 3, which is a drawing made from a section of liver of cat stained with eosine and methylene blue, shows two such cells. Red blood corpuscles are often seen lying between the processes of these cells and adjacent liver cells. Carmine gelatine when injected into the blood-vessels passes undiluted into the same position. It is difficult to determine whether any of the connective-tissue fibrils are continuous along the side of the projecting processes which is turned towards the wall of the vessel. They are not visible in ordinary preparations, and, if present, do not hinder the ready passage of injection mass and red blood corpuscles behind the processes. Cells of a similar nature are sometimes found lying between liver cells, but some portion of them always borders a blood-vessel and is in direct contact with the blood stream.

They are phagocytic, and frequently contain red blood corpuscles, either entire or in process of disintegration. We can find no evidence of their processes passing into the interior of adjacent liver cells in the manner described by Holmgren. Neither are we able to find any cells occupying the position in which Reinke described connective-tissue cells.

The livers of different kinds of animals vary to some extent in the appearance of the vessel walls; chiefly in the amount of connective-tissue fibrils present.

Whether the walls are composed of a syncytium or of an incomplete layer of cells, we are not in a position to state. In the animals we have examined the evidence seems to us in favour of the latter view. The connective-tissue fibrils do not hinder the passage of carmine gelatine from the blood-vessels into the liver cells, and in the dog's liver red blood corpuscles also pass into the liver cells. Whatever the composition of the wall of the blood spaces, assuming a wall to exist, it is very permeable and closely applied to the liver cells. The blood has an intimate relationship to the liver cells, and the exchange of material between the two takes place without the intervention of lymph spaces.

Kisselew (34) found lymphoid tissue inside the lobules of the pig's liver. The probability is that any lymphoid tissue found in the lobules, assuming it is not the result of tubercular or other pathological changes, is a secondary acquisition, and has passed in with the connective tissue accompanying the branches of the hepatic artery.

The Origin of Lymph in the Liver.

Kiernan (33) as long ago as 1833 recognised that when the portal vein is injected, the injection material soon appears in the lymphatics. This is easily verified in any well-injected preparation of the blood-vessels of the liver. Even if the injection is continued for a short time only, the large interlobular lymphatic trunks are distended with diluted injection mass; an injection continued for a longer time fills them with undiluted material.

The permeability of the vessel walls, which is so marked a characteristic of the intralobular blood-vessels, is also a feature of the blood-vessels at the junction of interlobular connective tissue and liver parenchyma. Starling (58) pointed out that the capillaries of the liver are the most permeable of all the capillaries of the body. He also showed that the lymph which comes from the liver contains from 6 to 8 per cent. proteids, almost as much as the blood plasma itself.

We believe that the lymph is collected at the periphery of the lobules in lymphatic spaces immediately surrounding the branches of the interlobular veins as they enter between the columns of liver cells; it passes into these spaces from the cells at the periphery of the lobules. In all probability the intracellular plasmatic channels of the liver cells act as an intermediate system linking the blood-vessels in the lobules to the lymphatics outside. If this is the case the plasma must flow from cell to cell on its way to the periphery of the lobule. In the injection preparations there is evidence that the channels do communicate from cell to cell, although it is difficult to wash the injection out of the cells by the perfusion of large quantities of salt solution through the blood-vessels. But injection of the lymphatics does not favour the idea that the intracellular channels open into them, for even where the interlobular connective tissue is full of injection material none passes into the liver cells at the periphery of the lobules.

How, therefore, the blood plasma passes from the cells into the commencing lymphatics must be left for the present undetermined. Whenever colouring matter which has been used in injecting the lymphatics is found in the liver cells, there is not the slightest doubt that the injection has passed into the cells from the interior of the blood-vessels, into which it has entered by rupture of their walls.

The large amount and the concentrated character of the lymph which comes from the liver must be ascribed to the peculiarities of the connection between the blood and liver cells and the sinusoidal character of the circulation in the lobules, the incomplete endothelium allowing the ready passage through it of fluid and even of small solid particles both into the liver cells and into the lymphatics.

Whether the endothelium possesses the power or not of altering the degree of permeability is uncertain. Mayer (44) believes that the walls of the liver capillaries can alter their permeability for fluid and solid constituents of the blood, and this quite independently of pressure changes or vaso-motor influences. The question is one which has an important bearing on theories of the formation of lymph, and requires further investigation.

If, as we believe, the liver cells lie in the direct path between blood stream and lymphatics we should expect that any agencies which increase the activity of the liver cell would also increase the amount of lymph flowing from the liver.*

Summary.

1. The liver cells are permeated by fine anastomosing channels which can be filled with injection mass from the blood-vessels. These channels undoubtedly receive plasma from the blood. In the dog, red blood corpuscles are occasionally seen within the liver cells, and crystals which closely resemble hæmoglobin are frequently found inside the cell nuclei. There must, therefore, be an intimate connection between the blood in the intralobular blood-vessels and the liver cells.

2. The lymphatics of the liver (dog, cat) are confined to the visible connective tissue of Glisson's capsule and the adventitia of the hepatic veins. The lymphatic vessels accompany the hepatic artery and its branches, forming networks around these vessels as well as around the branches of the portal vein and bile ducts. There are no lymphatics within the lobules. The perivascular lymphatics described by MacGillavry do not exist. Both portal and hepatic lymphatics leave the organ at or near the portal fissure.

3. The endothelium which lines the intralobular blood spaces (sinusoids in the sense of Minot) is incomplete and allows the passage through it both of fluid and of fine solid particles into the liver cells. The endothelial cells are of two kinds, large and small. The large cells (Kupffer's cells) are phagocytic, and often project into the blood spaces.

4. The concentrated character of the liver lymph is explained by the incomplete nature of the endothelium lining the intralobular blood-vessels, thus permitting the plasma to pass directly into the liver cells. It is possible that the cells of the lobule form a syncytium, and the lymph is thus able to pass from cell to cell. It is probably passed at the periphery of the lobules into the interstices of the connective tissue which lies between the lobules; here it enters the lymphatics. All conditions which would tend to promote the activity of the liver cells should, by virtue of these arrangements, also tend to promote the flow of lymph.

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* *Cf.* Asher, "Untersuchungen über die Eigenschaften und die Entstehung der Lymphe;" Asher and Barbera, 'Zeitschr. f. Biol.,' vol. 36, p. 154, 1898; Asher, 'Zeitschr. f. Biol.,' vol. 37, p. 261, 1898; Asher and Gies, 'Zeitschr. f. Biol.,' vol, 40, p. 180, 1900; Asher and Busch., 'Zeitschr. f. Biol.,' vol. 40, p. 333, 1900; Asher, 'Zeitschr. f. Biol.,' vol. 45, p. 121, 1904.

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DESCRIPTION OF PLATES.

PLATE 22.

FIG. 1.—Liver cells of normal dog. Eosine and methylene blue.

The upper part of the figure represents four liver cells, one with two nuclei. One of the nuclei contains a crystal, which has extended the nuclear membrane to more than twice the diameter of an average-sized nucleus.

The lower part of the figure represents two liver cells. The nucleus of one contains a shorter crystal, and its nucleolus lies to one side, close to the nuclear membrane and opposite the middle of the crystal. The cytoplasm of the same cell contains a semicrystalline body, which stains more lightly with eosine and resembles hæmoglobin. In the other cell a red blood corpuscle lies in the cytoplasm close to the nucleus.

Fig. 2.—Liver cells of normal dog. Eosine and methylene blue.

Four liver cells are shown, in three of which the nucleus contains a crystal. The crystal is of a different size in each. The nucleolus lies close to the nuclear membrane opposite the middle of the crystal. The nucleus of the fourth cell contains a rounded mass of a material which stains like the crystals; its nucleolus lies on one side, close to the nuclear membrane.

Fig. 3.—Part of a section of liver of cat. Eosine and methylene blue.

The sinusoidal character of the blood-vessels and the incomplete nature of their endothelial lining are seen.

One small endothelial cell is closely applied to the junction of two liver cells in the middle column. Two of Kupffer's cells are shown. They are large cells with processes and appear to lie free in the sinusoids. A space exists between each of them and the neighbouring liver cells. In the lower cell represented this space is partly occupied by red blood corpuscles. The cytoplasm of both cells contains granules staining intensely with eosine.

Fig. 4.—Section of rat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 80 mm. Hg; inferior vena cava open. The injection mass occupies channels between and inside the liver cells.

Fig. 5.—Section of rat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured.

The liver cells contain more injection mass than do the cells in fig. 4, but it is largely in the form of separate drops.

Fig. 6.—Section of monkey's liver. Hæmatoxylin.

The portal vein was injected with carmine gelatine at a pressure of 60 mm. Hg; inferior vena cava open. One of the liver cells contains a fine network of injection mass; in others there are small accumulations of the injection in the cytoplasm.

Fig. 7.—Section of dog's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured. There are channels filled with injection between and inside the liver cells.

Fig. 8.—Section of cat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured. The injection mass appears in fine channels in the cytoplasm of the liver cells.

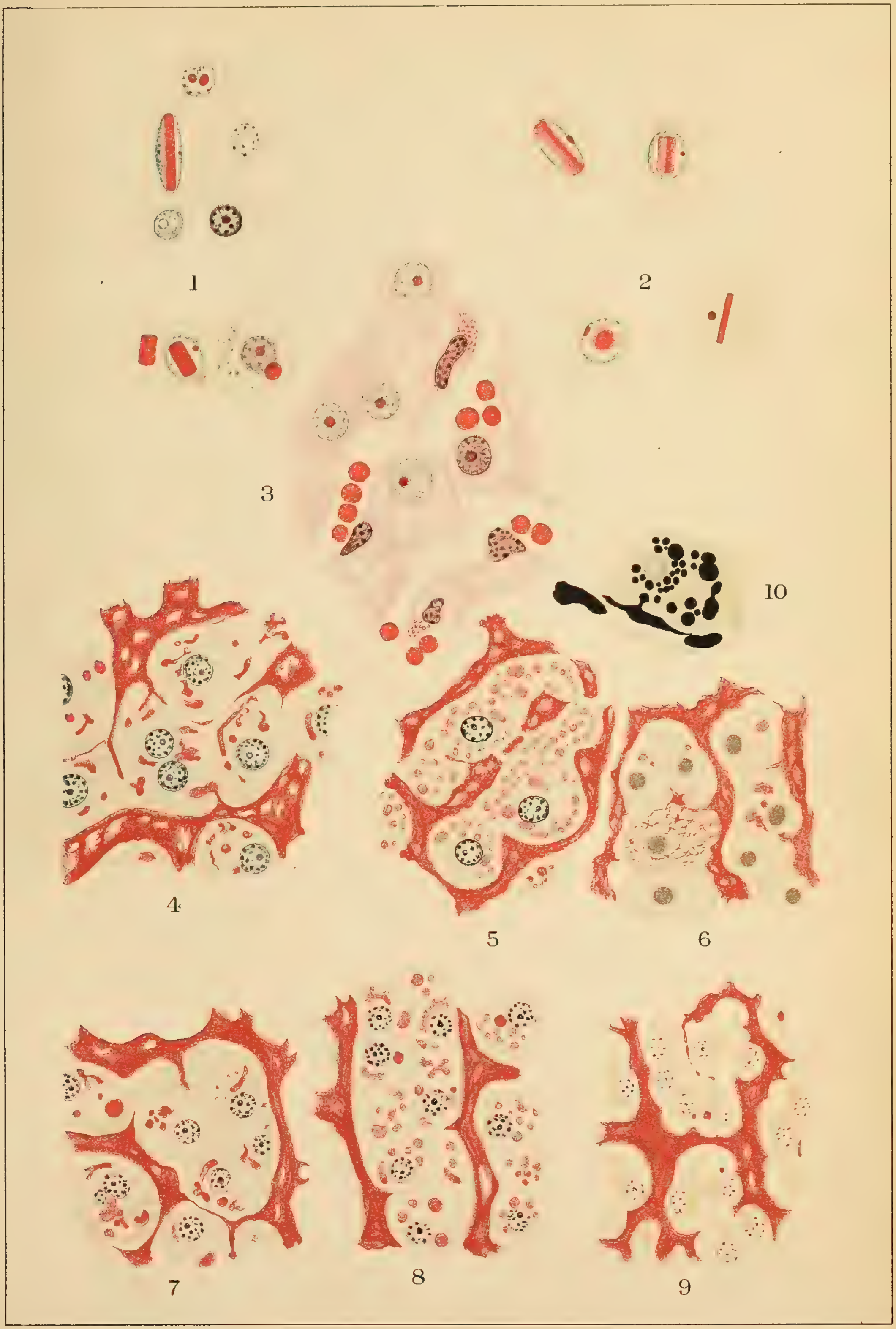
Fig. 9.—Section of fowl's liver. Hæmatoxylin.

Right aortic arch injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava open. The sinusoidal character of the blood-vessels is seen. Fine channels pass between some of the liver cells, and the cytoplasm here and there contains injection.

Fig. 10.—A liver cell and part of an adjacent blood-vessel from child's liver. Osmic acid and methylene blue.

From a case of fatty embolism due to chloroform poisoning.

The liver cell contains globules of fat, and a direct connection is apparent between the fat in the cytoplasm of the cell and fat in the blood-vessel.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Herring & Simpson. *Roy. Soc. Proc. B. Vol. 78. Plate 23.*

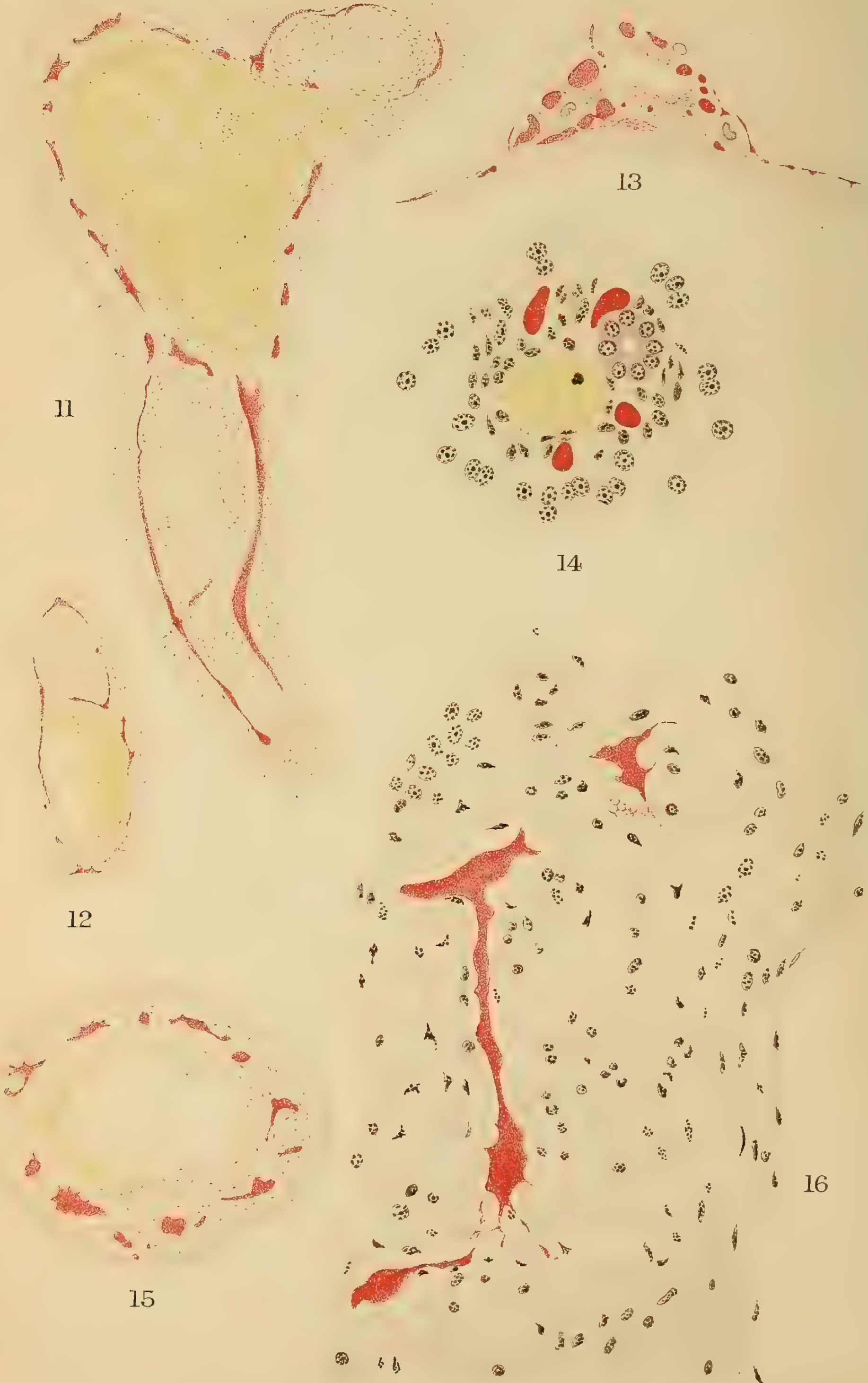
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PLATE 23.

FIG. 11.—Portal canal from cat's liver. Hæmatoxylin.

Lymphatics injected with carmine gelatine.

A network of lymphatics surrounds blood-vessels and bile ducts.

FIG. 12.—Small portal canal from cat's liver. Hæmatoxylin.

Lymphatics injected with carmine gelatine.

Shows a smaller portal space and the lymphatics occupying the connective tissue round the blood-vessels and bile ducts.

FIG. 13.—Portal canal attached to wall of hepatic vein from dog's liver. Hæmatoxylin.

Lymphatics injected with carmine gelatine.

Shows a large portal canal lying in the wall of one of the large hepatic veins. The inner surface of the vein forms the lower limit of the figure. The lymphatics of the portal space and those of the adventitia of the hepatic vein are seen to be continuous with one another.

FIG. 14.—A small portal canal from the dog's liver. Hæmatoxylin.

Lymphatics injected with carmine gelatine.

FIG. 15.—Hepatic vein of dog's liver in transverse section. Hæmatoxylin.

Lymphatics injected with carmine gelatine are seen in the wall of the vein. Small branches of the hepatic artery are also seen in its wall.

FIG. 16.—Longitudinal section of wall of a large hepatic vein in liver of dog. Hæmatoxylin.

Lymphatics injected with carmine gelatine.

Shows lymphatic trunks in the adventitia of the hepatic vein.

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